

THE AMERICAN JOURNAL OF PATHOLOGY

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The American Association of Pathologists and Bacteriologists*

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VOLUME XVII

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JANUARY, 1941

NUMBER I

MESODERMAL MIXED TUMORS OF THE BODY OF THE UTERUS *

AVERILL A. LIEBOW, M.D., and ROBERT TENNANT, M.D.

(From the Laboratories of Pathology of the Yale University School of Medicine, New Haven, Conn., and of the Meriden Hospital, Meriden, Conn.)

A new summary of anatomical and clinical data concerning mixed tumors arising in the body of the uterus is presented, together with experimental evidence concerning the histogenesis of these neoplasms obtained by the use of the tissue cultures. This comprises the first part of the paper. Three instances observed in this laboratory are related in the second part, and in the appendix appear brief summaries of the pertinent features of previously reported cases.

PART I

The diagnosis of malignant mixed tumor of the uterus is based upon the demonstration of unusual tissues as cartilage, bone or striated muscle. Other uterine neoplasms composed of malignant mesodermal elements include the leiomyosarcoma, endometrial sarcoma, carcinosarcoma and teratoma.

The status of the carcinosarcoma is uncertain. By this term is usually implied a tumor in which spindle-shaped elements are intimately intermingled with more or less typical epithelial cells and not one resulting from collision of a separate carcinoma and

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a sarcoma. These have been discussed in general by Claessen and Mathias and have been described in the uterus by Saltykow and by Manheims, among others. It is exceedingly difficult in many instances to state whether both stroma and epithelium have assumed malignant properties or whether the epithelial cells have merely become spindle shaped. In work with the transplantable tumors of mice, it has often been observed that an epithelial neoplasm may, with successive transplantations, come to resemble a sarcoma. Such for example was the fate of the tumor of the mouse now known as Sarcoma 37 and the same transformation may occur with pulmonary carcinoma of mice induced with dibenzanthracene (Andervont). An epithelial origin of such specialized mesodermal tissues as cartilage and striated muscle, however, is far more difficult to envision.

True teratomas of the uterus, containing derivatives of all three germ layers, have been reported only a few times; by Mann, by Hellendall, and by Lackner and Krohn.

Incidence

The authors have been able to find only 65 previously recorded instances of mixed tumors of the body of the uterus. Piquand discovered cartilage in 2 of 151 sarcomas of the body of the uterus. Among malignant neoplasms of the uterus sarcomas are variously stated to occur in a proportion of 1:40 (Bunten), 20:1000 (Mathias) and 46:1082 (Frankl). These differences probably depend upon the interpretation of the possible malignancy of tumors composed of smooth muscle. Most writers state that mixed tumors of the cervix are more common than those of the body, but Meikle is in disagreement. It is interesting that mixed cervical tumors were described earlier than those of the body of the uterus and that at the time of Wilms's monograph the former were already well known whereas the latter received no mention. If one accepts the figures of Piquand and Mathias, 1 in every 7,500 malignant tumors of the body of the uterus is of this variety. It is probable that many instances have been missed because of the difficulty of demonstrating striations in the muscle fibers.

TABLE I
Modes and Sites of Origin

	Diffuse: obliterating uterine cavity	Arising diffusely as papillary or botryoid masses	Localized origin Pedunculated or sessile masses			Exact manner of origin not stated
			Posterior wall	Cornu and lateral wall	Anterior wall	
Fundus		Gamper	Frankl (case 3) Glynn and Bell (case 1) Glynn and Bell (case 2) Gunning and Ross Halter von Franqué	Amolsch Herb Reeb and Oberling	Hunziker Lochrane	Bystroumow and Eckert Frank Liebow and Tennant (case 3) Shapiro
Cervix and fundus		Läwen				Anderson and Edmansson Colomiatti Robertson
Fundus	Hartfall (case 2) Jessup Kleine Sophian (case 2) van Akkeren	Frankl (case 1) Köhler Murray and Littler Perlstein Wiener	Azzola. Chavannaz and Nadal (case 1) Chavannaz and Nadal (case 2) Hartfall (case 3) Liebow and Tennant (case 1) Liebow and Tennant (case 2) Olander Rankin and Broders (case 1)	Gaebelein Gebhard Geisler Hartfall (case 1) Hofbauer Kistler Lahm Reinecke	Fels Penkert	Blasek (case 1) Blasek (case 2) Delagenière and Beauchef Frankl (case 2) Kaufmann (case 1) Kaufmann (case 2) McDonald, Broders and Counseller Nicholson Petersen (case 1) Petersen (case 2) Reid Schröder and Hillejahn Sophian (case 1) Stout Wagner Wolfe
Cervix and fundus						Durante and Roulland Malapert and Morichau- Beauchant Seydel

Containing striated muscle

Containing cartilage and other tissues

Pathology

In discussing all available material it is necessary to distinguish such tumors as arise solely from the fundus from those involving also the cervix until it can be established whether or not they are fundamentally different. Furthermore, it may be well to make a grouping depending on manner of growth and site of attachment. As to the former it must be considered whether the neoplasm arose diffusely, obliterating the uterine cavity; whether it was multicentric, having a papillary or botryoid character; or whether it was polypoid. These groups in turn may be subdivided on the basis of the nature of the constituent cells. It seems reasonable to assume, as will be indicated, that tumors containing striated muscle are least likely to result from metaplasia of preëxisting elements and therefore demand some other explanation. Next in order of probability of nonmetaplastic origin are the tumors composed of several tissues, some heterotopic.

An analysis of Table I, which was constructed with these principles in mind, reveals that most of these tumors are polypoid and attached either in the region of the cervix or upon the posterior wall of the fundus. They often traverse the cervical canal and enter and even distend the vagina as partially necrotic and hemorrhagic masses yielding a sanguineous, serosanguineous or foul seropurulent discharge. Relatively few arise as multiple papillae or diffusely.

Varieties of Tissue

The types of tissue contained are indicated in Table II. Subdivision is made depending on whether striated muscle or cartilage was the predominant constituent. Most of the tumors contained cartilage and undifferentiated elements with or without other tissues. It is interesting to note that only four in the group included both cartilage and striated muscle.* Tumors consisting chiefly of striated muscle were more rarely mixed with other tissues than were those composed chiefly of cartilage. In both groups epithelium of various types was the most frequent additional tissue. In some instances the epithelium did not have the characteristics of malignancy, but in others there was carcinoma, either glandular

* Hunziker, Gamper, Frankl and Amolsch.

or undifferentiated. Bone or osteoid tissue and smooth muscle came next in frequency. Nervous tissue was identified only twice, by Schröder and Hillejahn, and by Kleine.

TABLE II
Distribution of Tissues in Types of Tumors

Varieties of tissue	Grouping		Total
	Tumors composed chiefly of striated muscle	Tumors composed chiefly of cartilage	
Striated muscle	20	...	20
Cartilage	4	42	46
Myxosarcoma or undifferentiated sarcoma	12	36	48
Giant cells	6	7	13
Epithelium	7	19	26
Smooth muscle	2	5	7
Nervous tissue	...	2	2
Fat	...	3	3
Osteoid or bone	2	3	5
Endothelium	...	2	2

Recurrences and Metastases

Of 68 cases (including the 3 described in this paper) the fate of only 28 patients is reported. One patient (Hartfall, case 3) was in good health 5 years after operation. Another (Petersen, case 2) who had a tumor consisting largely of fat, was well 2 years after hysterectomy and 6 years after onset of symptoms. Kleine's patient was apparently well 2 years postoperatively and Gamp-er's, 4 years. Five others, examined clinically from 3 months to 1 year after operation, seemed free of recurrence (Halter; Liebow and Tennant, case 3; Kistler; Sophian; and McDonald, Broders and Counseller). The other 19 patients all succumbed. Four died in the period immediately after operation. Three of the 19 showed no metastases at the time of operation and 1 was without metastases when examined at necropsy. The remaining 16 all died with metastases but necropsies were available only on 4. All of these 4 * had metastases to the lungs or pleura. It is probable that metastases to the thoracic viscera were much more frequent than was apparent merely from the clinical reëxamination of the patients, especially when roentgenograms were not available. Considering all of the 19 deaths, local recurrences were most commonly found (15 of 19), sometimes with further extension into

* van Akkeren, Hartfall (cases 1 and 2) and Wagner.

adjacent peritoneum resulting in compression of the intestines or veins. One patient, reported by Delagenière and Beauchef, had a tumor of the tibia. When the extremity was amputated, the tumor was found to have the structure of a sarcoma. It was not certain whether this was a metastasis.

Metastases were of three varieties:

1. The tissues of the primary lesion and metastases were the same.
 - a. Hartfall (case 1): Myxomatous tissue and cartilage
 - b. Hartfall (case 2): Myxomatous tissue and cartilage
 - c. Wagner: Cartilage
2. Only some of many varieties of tissue were found in the metastases.
 - a. Hunziker: *Primary*: striated muscle, cartilage, round and spindle cell stroma
Metastases: striated muscle
 - b. Fels: *Primary*: sarcoma, cartilage, epithelium
Metastases: epithelium
 - c. Liebow and Tennant (case 2): *Primary*: epithelium, sarcoma, cartilage
Metastases: myxosarcoma, epithelium
3. Only dedifferentiated tissue was found in the metastases.
 - a. van Akkeren: *Primary*: cartilage
Metastases: giant cells
 - b. Glynn and Bell (case 2): *Primary*: striated muscle
Metastases: myxosarcoma

Pathogenesis

The origin of malignant tumors composed of heterologous tissues has been the subject of much speculation.

There are some who consider them to be malignant growths of metaplastic origin. Cartilage and bone are indeed sometimes the result of metaplasia. The former may develop in myxomatous connective tissue; the latter is often found where cartilage has been before as in the bronchial rings or in dense connective tissue as, on occasion, in the substance of uterine fibroids. Pierson has found that cartilage may develop in the stroma of the rabbit's

uterus under prolonged estrinization. It is more difficult to explain the presence of striated muscle on the basis of metaplasia. More specifically, there is no evidence that striated muscle can develop from smooth muscle. In the phrase of Wilms, "striated muscle has the same relation to smooth muscle as it does to cartilage or fat."

The possibility that malignant tumors may arise from the rare benign neoplasms of heterologous tissue (see Ascher, Kworostansky, Feuchtwanger, Pietzold and others) has been discussed. Again the origin of these remains obscure. Simple heterotopic inclusions not in the form of tumors are exceedingly infrequent. Thus Meyer (1930) reported the presence of a nodule of bone in a fetal uterus and of normal cartilage in the genitalia in extra-uterine position. He considered these to be the result of development of fetal inclusions, however, rather than of metaplasia. Striated muscle was found in a postpartum uterus by Nehrkorn and by Girode. Blasek has described a nodule of typical cartilage in the stroma of a fragment of otherwise normal endometrium.

The multiplicity of tissues in so many of the malignant heterologous tumors makes the theory of their development from pluripotential anlagen seem relatively attractive. A true teratoma with organized arrangement of tissues from all three germ layers has been found only a few times. The source tissue is thus multipotential rather than totipotential. In his second monograph (1900) Wilms adduced evidence in support of a cell-rest theory of origin of tumors of the lower genito-urinary tract as he had done previously for the mixed tumors of the kidney (1899). Mixed tumors of the vagina, cervix, vas deferens and bladder were discussed but not those of the body of the uterus. Inclusion of myotome and sclerotome or of the predecessors of these from the dorsal segments of the posterior portion of the body was held responsible for mixed tumors of the lower generative tract. Among others, Seydel and Meyer (1903) amplified the theory by suggesting the possibility of displacement of anlagen in various stages of differentiation during the growth of the Wolffian duct or its derivatives. Seydel presented a diagram to illustrate how a portion of the blastema might come to lie ventral to the Wolffian duct and thus be displaced. More recently Masson, who restudied the embryonal adenosarcomas of the kidney, has demonstrated

an abundance of nervous tissue within them and has indicated the probability of their derivation from primitive neuro-epithelium. This neuro-epithelium may have as its derivatives nervous tissue, "mesectodermic" elements, certain muscles and the nephrogenic mesenchyme. The capacity of the neural crests to give rise to connective tissues had previously been demonstrated by Stone who called these tissues "mesectoderm." What rôle neuro-epithelium plays in the origin of mixed tumors of the uterus remains obscure. Certainly the content of nervous derivatives in our first case is exceedingly small. Whatever the exact genesis of the tumors, the idea of pluripotential anlagen makes superfluous the once current nosological division into subgroups depending on morphology.

Reticulum stains performed on sections from case 2 of our series demonstrate the disposition of argyrophil fibers about the various constituents of the mixed tumor in a manner characteristic of epithelium, cartilage and sarcoma respectively (Figs. 11 and 12). The relation to the cartilage was as in material similarly stained from a case of chondrosarcoma of bone, *i.e.*, the fibers either ran parallel to the cartilaginous mass or seemed to enter at an angle to become lost in the hyaline matrix.

An experimental approach was available in studying the first of our tumors. This consisted of explanting the tissue into culture media in Carrel flasks. Fragments of firm, translucent tumor tissue obtained under aseptic precautions through a seared surface resulted in a growth of cells closely resembling that of embryonic human cartilage (Figs. 5-8). In both instances, cells grew from the first explants in columns which did not branch immediately. Daughter cells within any column tended to remain adjacent, trellised upon one another and intertwined, rather than to branch out at an angle. In this respect they differed from cultures of fibroblasts. Distally the cells became shorter and shorter and the terminal cell was a blunt, rounded element with a flame-shaped, free border. The tumor cells were larger than those of normal fetal cartilage. Coarser and more abundant vacuoles encircled the nucleus, which had a prominent membrane and nucleoli. Whatever the origin of these tumor cells, (whether metaplastic or dysembryogenic), they had *in vitro* many characters in common with normal cells of fetal cartilage.

Clinical Notes

The sharp difference in age incidence between mixed tumors of the vagina, of the cervix and of the body of uterus has been discussed by many authors and has been illustrated graphically by

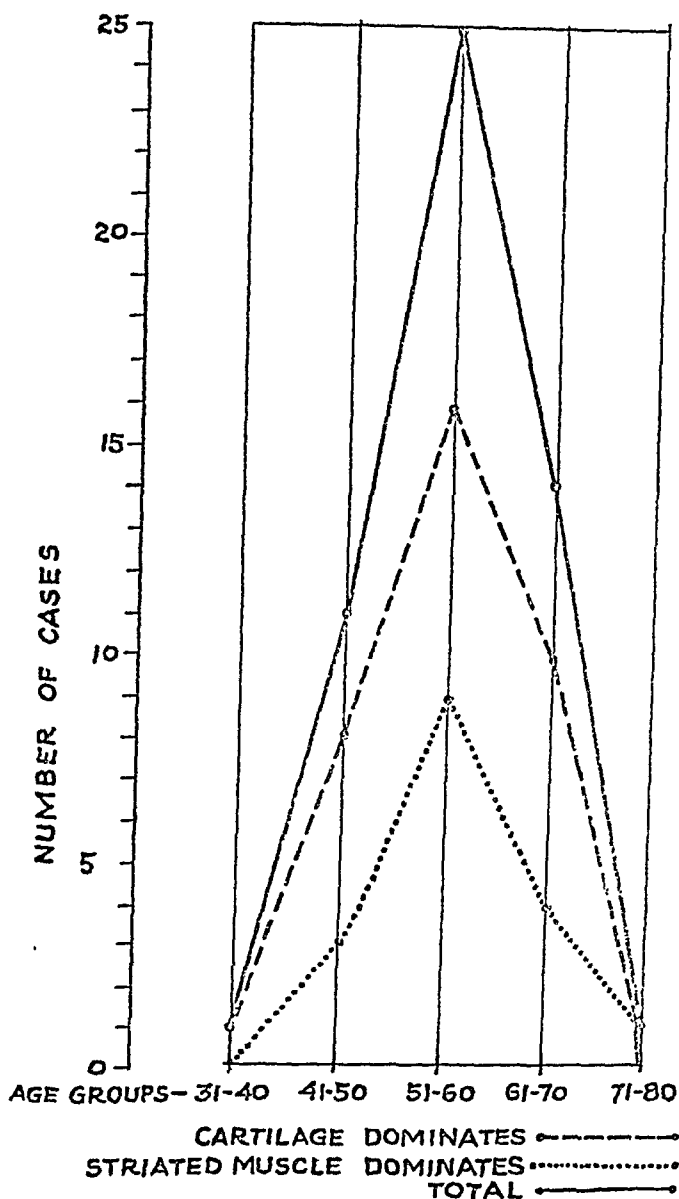


Chart 1. Distribution of all cases, of those in which cartilage dominates and of those in which striated muscle dominates, according to age groups.

Meikle. The curve obtained by plotting the data from the larger material relative to mixed tumors of the uterine body as surveyed here is presented in Chart 1. The sharp peak of the curve at

about 56 years of age is striking. Twenty per cent of the patients had never borne children.

Clinically, these tumors almost always manifest themselves by a sanguineous discharge from the vagina. Only 6 of 47 patients did not have this symptom. Five had as their chief complaint pain in the lower abdomen. Eleven others had this as a major complaint. This is noteworthy since pain is a much less common symptom with carcinoma of the fundus. The pain was described as aching, cramplike or, in 1 patient, like that in labor. In 2 others it was combined with dysuria. Perhaps pain is associated with the bulky, polypoid nature of many of these tumors about which the musculature of the uterus may contract. Two patients were conscious of an abdominal mass and 1 complained of an increase in the size of the abdomen. Three, all with mixed tumors composed largely of striated muscle, had passed portions of the tumor *per vaginam*.

The average duration of symptoms was about 29 weeks in the 29 patients from whom this information was available.

Only 4 patients upon whom follow-up data were available survived more than 2 years. The others died on an average of 33 weeks after the diagnosis was made and 52 weeks after onset of symptoms. This indicates the inadequacy of even the radical hysterectomies that were performed upon almost all of these patients.

PART II

CASES COMING UNDER THE OBSERVATION OF THE WRITERS

Case I

C. C., a white American housewife, 66 years old, was admitted to the New Haven Hospital on December 30, 1936 complaining of vaginal bleeding. This had started 6 months previously and had continued intermittently two to three times a week up to the time of admission. The bleeding was never excessive and had not necessitated the wearing of a pad. During this period the patient felt run down, tired easily and lost 20 pounds in weight.

The menses, which started at the age of 13, came regularly at monthly intervals and lasted 5 days. Menopause occurred at the age of 48 and the patient was free of symptoms until the onset of the present illness in August 1936. The patient was never pregnant although contraceptives were never employed.

The patient was moderately obese and had a blood pressure of 180/100. There was evidence also of cardiac hypertrophy. The lungs were clear to auscultation and percussion. Vaginal examination revealed a soft, smooth cervix with an os which admitted the tip of the finger. The body of the uterus was increased in size but the adnexa were not felt.

On January 4, 1937 the uterus was curetted. The cervix was smooth and devoid of ulcers. The uterus was retroflexed and definitely enlarged. A thickening suggestive of an old inflammatory process was felt in the adnexal regions. The cervix was dilated and the uterine cavity was found to measure 11.4 cm. in depth. Exploration of the cavity with a blunt, serrated curette revealed a zone of softening in the region of the right cornu and a similar less extensive zone on the left. The lining of the remainder of the uterus had a normal consistency. The curettings obtained consisted of vascular, friable tissue definitely suggestive of neoplasm.

The histological diagnosis of the tissue was leiomyosarcoma. Roentgenographic examination of the chest on January 7, 1937 revealed several small, metastatic tumor nodules in both lung fields. On January 18 a total hysterectomy was performed. The uterus was found to be five times normal size and apparently contained a myoma in the fundus. The left ovary was enlarged, cystic and adherent to the pelvic peritoneum. The right ovary was atrophic. The patient was discharged from the hospital on February 3 symptomatically improved. A roentgenogram of the chest 2 days before discharge showed extensive spread of the pulmonary metastases.

At home the patient improved subjectively although she expired 4 months after the onset of disease, apparently from extensive metastatic tumor. Post-mortem examination was not obtained.

Gross Notes. The surgical specimen consisted of the entire uterus including the cervix, tubes and ovaries (Fig. 1). The uterus measured 9 cm. in width, 9 cm. in length and 6 cm. antero-posteriorly in the fundic portion, which appeared to be markedly enlarged. The serosal surface was smooth and shining except for a solitary myoma 1 cm. in diameter on the posterior wall immediately above the peritoneal reflection in the cul-de-sac. Both tubes were thin walled and their fimbriated ends were free. The right ovary was a small, firm, yellow, almond-shaped, atrophic organ which measured 3 by 1.5 by 0.8 cm. The left ovary was an enlarged cystic spherical mass which measured 5 by 3.5 by 3.5 cm. Its serosal surface was smooth and shining but somewhat irregular. On cross section this ovary appeared to be completely replaced by soft, yellow, friable tissue forming tiny papillary structures that completely filled the lumen of a cyst. Surrounding the cyst was firm, homogeneous, yellow, fibrous tissue. The left fallopian tube was attached along the surface of this cystic mass. When the uterus was sectioned a large mass was found to occupy

the fundic portion. This replaced most of the uterine musculature in the posterior wall and appeared to arise from almost the entire fundus and posterior wall. It did not appear to extend appreciably into the anterior wall. The mass within the wall measured 4.5 cm. in diameter. It extended into the lumen of the uterus as a polypoid structure which measured 3 cm. in length and 2.5 cm. in diameter. On the lateral walls the polypoid projection was adherent at several points. The apical portion of the mass had a gray brown, opaque, friable surface. In gross appearance the tumor resembled a polypoid submucous myoma. It differed, however, in that it was adherent to the wall at several points and the line of demarcation between the myometrium and the tumor itself was poorly defined. On cross section the tumor presented a surface of interlacing strands of dense white tissue interspersed with a more gelatinous, translucent tissue. The uterine wall in the fundus was thinned to a width of approximately 0.5 cm. in contrast to uninvolved portions which measured approximately 1.5 cm. in thickness. The endometrium below the tumor had a smooth, shining appearance. The cervix was smooth, firm and covered by intact epithelium.

Microscopic Notes. The tumor, microscopically, was found to be composed primarily of cartilaginous tissue in varying degrees of differentiation (Figs. 2-4). It was made up of masses of this tissue separated from one another by strands of spindle-shaped cells which appeared to represent remnants of the myometrium. This cartilage-like tissue had a matrix composed of homogeneous blue staining material, scattered throughout which were cells with basophilic, vesicular nuclei and vacuolated cytoplasm. In many instances these cells were arranged in pairs and the general appearance suggested that of adult cartilaginous tissue. As one approached the periphery of such masses, the blue matrix disappeared and dense cellular tissue was encountered (Fig. 2). The transition from the central to the peripheral portion was gradual. The cells at the periphery were large, and varied from polyhedral to spindle shaped. The nuclei were large, blue staining and vesicular with only a loose reticular framework. The cytoplasm was basophilic. In this peripheral portion numerous cells were found in mitotic division. The matrix in several of the masses stained a deep blue or purple and had a granular appearance indicating

calcification. In some places a pink staining quality of the matrix suggested osteoid tissue although no well formed, bony structure was encountered (Fig. 3). At the periphery of the masses the tumor cells extended in irregular fashion into the surrounding myometrium. In several places at the periphery the tumor lay within endothelium-lined channels which represented either lymphatics or small blood vessels (Fig. 4). The free surface of the tumor which projected into the uterine cavity was necrotic and was covered by an exudate of polymorphonuclear leukocytes. These infiltrated for a short distance into the tumor. There was also extravasation of blood in this portion. The endometrium was completely absent over the distal part of the tumor, and throughout the uterus it was atrophic and composed of occasional simple glands, some of which appeared dilated and lined by a columnar epithelium with basally placed nuclei. The endometrial stroma was almost entirely lacking. The endometrium was reflected onto the surface of the tumor but was at once interrupted by necrosis and ulceration. The myometrium was atrophic, and consisted of small spindle-shaped cells containing small, deeply staining nuclei.

The cyst of the left ovary possessed a dense wall of fibrous tissue about which the usual spindle-shaped, deeply blue staining cells of the ovarian stroma could be identified. The cyst was lined by innumerable papillary projections that branched in complicated fashion to fill the entire lumen. The papillary projections had delicate fibrous stalks covered by a variable, tall columnar epithelium. Many of these cells were in mitotic division. In some portions the epithelial cells appeared to infiltrate the fibrous tissue of the wall irregularly. Extensive necrosis of the epithelium had occurred in several places and here only a granular, amorphous, pink staining debris remained. In other portions fusion of numerous villi gave the tumor an acinar appearance. The fallopian tubes were atrophic structures with simplified villi composed of a central fibrous stalk covered by columnar epithelium.

Discussion. The cartilage resembled closely that of other tumors. Its growth properties *in vitro*, as described in the text, were very much like those of fetal cartilage (Figs. 5-8). This case is also of interest because there was an associated papillary cystadenocarcinoma in the left ovary.

Case II

This patient was a dressmaker of French-Canadian descent and 59 years old at the time of her first admission to the Meriden Hospital. Her chief complaints at this time were of pain in the lower abdomen and of bleeding *per vaginam*. Her menopause had come at 47 years of age, after a normal menstrual life beginning at 13. There was no history of serious illness in the past nor had any operations been performed. Two and one-half months before admission the patient began to have pain in the lower abdomen. The pain was intermittent and was associated with a scanty, sanguineous, vaginal discharge. The attacks gradually became more frequent and the pain more severe until 1 week before entrance into the hospital, when both pain and discharge became continuous.

Physical examination showed nothing of note except a blood pressure of 200/100. Vaginal examination revealed a bleeding, partially necrotic mass extending through the external os. The attachment was in the fundus. The uterus was not enlarged.

On October 11, 1937 the polypoid mass was excised by cutting the pedicle with scissors. When the diagnosis of malignant mixed tumor of the uterus was made, the patient was urged to return for hysterectomy. The uterus, cervix and adnexa were extirpated on November 15. Postoperative recovery was uneventful. She was readmitted on December 19, 1938 complaining of abdominal pain. She had felt well until about 5 weeks before this time, when she was seized with cramplike pains in the abdomen. Her abdomen became distended and she felt nauseated. The chest was clear to percussion and auscultation. In the left flank of the distended abdomen was felt a large mass. Another firm mass was palpated to the left of the vaginal vault. At the exploratory laparotomy that was performed on December 20, numerous exceedingly firm nodules were found in the floor of the pelvis and within the mesentery and omentum. The latter was thick, extended boardlike across the abdomen and was adherent to the liver and adjacent loops of intestine. A nodule was removed from the omentum for histological examination. This contained carcinoma and myxosarcoma but not cartilage. The patient died at home on January 22, 1939. Necropsy was not performed.

First Specimen

(Polypoid mass excised October 11, 1937. M.S.P. No. 4583.)

Gross Notes. The specimen consisted of two masses of tissue. The larger was mushroom shaped and measured 5.5 by 4 by 2.5 cm. The smaller was ovoid with dimensions of 3.5 by 2.5 cm. That part of the larger specimen which corresponded to the head of the mushroom resembled the smaller mass in its gross features. The tissue was soft and was composed of a yellow gray opaque matrix enclosing numerous translucent gray zones which did not exceed 3 mm. in diameter. Hemorrhage had occurred in a small region near the periphery and a rough, irregular dark brown clot was visible on the surface. Minute depressions could be distin-

guished on the cut surface, possibly corresponding to the lumina of glandular structures. The stalk of the mushroom consisted of closely appressed nodules of firm tissue that gave the specimen a bosselated appearance. Within an extremely translucent gray matrix lay whorls of opaque white fibers. This tissue resembled a common fibroid.

Microscopic Notes. Histologically, the protruding mass was remarkable for the diversity of its component elements (Figs. 9-13). There were acini of various types, scattered round and spindle-shaped cells resembling those of sarcoma, and islands of tissue with the characteristics of cartilage. Striated muscle fibers were not found in an extensive search.

The acini, which had no resemblance to the uterine glands, varied in size and shape as did their lining cells. These were cuboidal or columnar cells devoid of cilia. Some of the smallest acini were lined by flattened cells but keratinization was not in evidence. The lining cells as well as those of the interstitium were often found in a state of mitosis and these mitoses frequently were atypical. Only occasionally were eosinophilic nucleoli seen. Chromatin in all cells of the tumor occurred as delicate strands except in a few pyknotic elements. Certain of the interstitial cells had huge polymorphous nuclei within a vaguely defined cytoplasmic mass. The islands of cartilage had, superficially at least, a typical appearance. The matrix was homogeneous and stained deep blue with hematoxylin and green with Masson's Lichtgrün stain. The small nuclei of the cells within this matrix were surrounded by vacuolated, basophilic material. At the margin of the islands spindle-shaped cells grouped themselves concentrically in successive layers. Internally they shaded imperceptibly into the cartilage-like cells. Externally they resembled more and more the interstitial cells. In some places, however, cartilage was not so sharply defined. It appeared to be merely a development of a matrix substance that widely isolated certain elements of the interstitium.

In the junctional zone of preparations stained by the Wilder method the reticulum was found to form a sharp basement membrane for the epithelium but not for the cartilage (Figs. 11 and 12). In the case of the latter, reticulum fibers seemed to fade into the cartilage matrix, often entering perpendicularly to the expected

course of the fibers in a basement membrane. The reticular support of the interstitial elements was very dense and embraced each cell in many places but often small groups of cells were isolated, suggesting that much more of the interstitium may be epithelial than seemed probable at first glance. There was variation in different sections in the proportions of cartilage, acini and interstitium. In some places there was infiltration with small mononuclear cells. Proximally the atypical tissue was not sharply delimited from tissue that had the histological appearance of a myoma. Deeper within the latter there were spaces lined by epithelium resembling very closely the acini seen in the tumor proper. On its external aspect the tumor tissue had become necrotic and was densely infiltrated with polymorphonuclear leukocytes.

Second Specimen

(Excised November 15, 1937. M.S.P. No. 4638.)

Gross Notes. This specimen consisted of a uterus removed intact with cervix and adnexa. It was a small, thin-walled, pear-shaped structure measuring 7 cm. from the serosal aspect of the fundus to the external os, 3.5 cm. transversely and 1.5 cm. anteroposteriorly. All of the serous surfaces were smooth and transparent. The thickness of the myometrium did not exceed 1.5 cm. It consisted in general of a gray, translucent and resilient, fibromuscular tissue embedding thick, firm-walled blood vessels. It was lined for the most part by a shining, exceedingly thin, mucus-covered, gray pink membrane. On the posterior aspect, beginning about 1 cm. from the ostium of the right tube, was an oval, rough, red, elevated zone projecting about 2 cm. into the lumen. A much smaller rough area was found in the wall of the cervical canal posteriorly. Sections were made including these two portions of the uterus. Each ovary was an ovoid structure 2.5 by 1.5 by 1 cm. The capsules were smooth. There was a translucent stroma enclosing many convoluted, more opaque corpora fibrosa. Each tube pursued a slightly convoluted course, was 6 cm. long and terminated in free fimbria.

Microscopic Notes. A section was examined from the wall of the uterus at the elevated, rough, red zone described grossly. This was the apparent site of origin of the tumor. Here a lining mem-

brane consisting of a single layer of tall columnar epithelium was made to project into the lumen by clublike extensions of a rather loose stroma of connective tissue containing dilated capillaries, extravasated red blood cells, and small mononuclear cells. Into the stroma projected irregular glands but the epithelium was only slightly atypical and mitoses were few. The remarkable thing was that such small atypical glands were situated within their myxomatous stroma deep within the myometrium. Often minute buddings of the glands occurred and in other sections from the same region a thick papillary layer of glandular tissue was found. This resembled, in some respects, the epithelium of the cervix; the cytoplasm was abundant and vacuolated and the cells were tall and the nuclei in general had a basal position. The adjacent endometrium was atrophic, the glands were typical but small, and the stroma consisted of dense, spindle-shaped, deeply staining, basophilic elements.

Third Specimen

(Fragment of omentum removed at exploratory laparotomy December 20, 1938. M.S.P. No. 5574.)

Gross Notes. The specimen consisted of a mass of tissue said to be derived from the omentum. Most of it was translucent yellow adipose tissue embedding very firm, gray, translucent masses. The latter offered great resistance to incision.

Microscopic Notes. In the mass removed from the abdomen the tissue was very much like that of the deeply invading glandular tissue of the uterus, but here the acini were more atypical (Fig. 13). These were again embedded in the myxomatous stroma which in turn was surrounded by dense collagen. Mitoses were not common. Although the stroma was myxomatous, actual cartilage was not in evidence.

Discussion. In preparations stained by the Wilder method, epithelium, sarcomatous stroma and cartilage maintained typical relations with the reticulum. That the original tumor and metastases may differ has often been noted.

Case III

The patient was a white woman, 62 years of age, upon whom there was performed an hysterectomy under suspicion of chorionepithelioma. Only

meager clinical data were available. It was stated that for 3 months she had been troubled with a bloody vaginal discharge, severe backache and pain in the lower abdomen. She had her menarche at the age of 16 years and the menopause had occurred suddenly at the age of 55. There had been one miscarriage, but four children were delivered normally.

Bimanual palpation showed the uterus to be firm and about six times the usual size. After removal it was described as soft and boggy. The tumor was stated to have been attached to the fundus.

Gross Notes. Only a small part of the tumor measuring 4 by 5 by 3 cm. was sent to the laboratory for study. About one half of the surface of the specimen was smooth and shining but beneath this surface could be seen extravasated blood. Incision showed the tissue to have a faint pink color and to be traversed by whorls of fibers. All cut surfaces had the same appearance.

Microscopic Notes. Microscopic examination showed the tumor to consist of polymorphous elements (Figs. 14 and 15). These were largely spindle-shaped cells that varied greatly in size. They occurred in interlacing fasciculi. There were large, apparently empty spaces among the cells, probably the result of shrinkage because of poor fixation. Sections stained with Sudan III showed only scanty deposits of fat in the form of fine, intracytoplasmic granules. A few of these cells showed distinct cross striations, particularly in preparations stained by the Wilder silver stain. Such striations were also found in some of the many mononuclear giant cells that were scattered among the spindle-shaped elements. Here the striations formed remarkably complex patterns (Figs. 16-20). The long, vesicular nuclei of the giant cells resembled those of the other cells. Their chromatin was in the form of minute granules which condensed at the periphery to form a distinct but thin nuclear membrane. Single or many brightly acidophilic nucleoli were surrounded by narrow halos of clear nucleoplasm. Mitoses were moderately abundant and often atypical. A few acini lined by cuboidal cells contained a finely granular material that stained with eosin (Fig. 14). Wilder's reticulum stain demonstrated a sharp, delimiting argyrophil membrane upon some aspects of these acini, but elsewhere the lining cells were in contact with, and difficult to distinguish from, the surrounding polymorphous elements. Reticulum fibers occurred also in small wisps intimately enmeshing individual cells elsewhere. There was an abundance of collagenous stroma throughout, as demonstrated in

preparations stained by Masson's Lichtgrün method. Small, poorly demarcated foci of necrosis were seen in a few places but hemorrhages were few. The abundant blood vessels had thin walls and were lined in some places by typical thin endothelial cells, but elsewhere by large cells indistinguishable from those of the tumor. The methods of Bielschowsky and Nissl failed to demonstrate nerve cells in the tumor.

Discussion. This tumor consisted of sarcoma-like tissue, striated muscle cells and epithelium. The last was in some places vaguely delimited from the first, suggesting the possibility of a common derivation in the sense of Masson. Evidence for this was not conclusive. There were no structures undeniably similar to those of the neural crest, and nerve cells could not be demonstrated.

SUMMARY

Mesodermal mixed tumors are among the rarest and most malignant neoplasms of the body of the uterus. They occur almost entirely in women between 45 and 65 years of age and manifest themselves clinically as does carcinoma of the uterus except that abdominal pain is more often a major symptom. Most of these tumors are polypoid and usually take origin either at the cornua or from the posterior wall. Metastases are most often local. No essential difference is noted between tumors containing striated muscle and those consisting in part of cartilage.

The mixed tumors present histological features typical of the various tissues which compose them when studied by means of reticulum and other special stains. In tissue cultures a cartilaginous tumor grows in a pattern characteristic of normal cartilage. These observations, together with the multiplicity of the tissues in many of the tumors, support the theory that pathogenesis depends upon multipotential anlagen rather than upon metaplasia.

APPENDIX

CASES PREVIOUSLY REPORTED

Mixed Tumors Composed Chiefly of Cartilage or Bone

- Azzola, Fabian. Ein Fall von Sarcoma uteri polymorphocellulare. *Zentralbl. f. Gynäk.*, 1924, 48, 2285-2287.
Age 47. Prolapse of uterus, pain in back and frequent and painful urination, 6 to 7 weeks. Bosselated tumor, size of child's head, embedded in

posterior wall of uterus. Histology: Islands of cartilage invading veins; polymorphous elements, including giant cells.

Blasek, Stefan (case 1). Knorpeleinschlüsse in der Uterusschleimhaut. *Arch. f. Gynäk.*, 1930, 141, 539-547.

No clinical data. Histology: Adenocarcinoma, myxomatous stroma, cartilage.

Blasek, Stefan (case 2). *Ibid.*

No clinical data. Histology: Adenocarcinoma, myxomatous stroma, cartilage.

Chavannaz, M. M., and Nadal, Pierre (case 1). Des tumeurs mixtes de l'utérus. *Gynécologie*, 1920, 19, 3-35.

Age 52. Severe pain in right side of abdomen. Enlarged soft uterus adherent to peritoneum. Springing from left posterior aspect of uterus, apparently from the musculature, was a tumor, size of a fetal head at term. Histology: Sarcoma, angiosarcoma, islands of cartilage, osteoid tissue, adenomyoma, multinucleated giant cells. Death $3\frac{1}{2}$ months after hysterectomy.

Chavannaz, M. M., and Nadal, Pierre (case 2). *Ibid.*

Age 59. Ascites and mass in abdomen, abdominal pain, vaginal bleeding. Unusual nodule arose from posterior wall of uterus. Histology: Cylindrical epithelial cells, sarcoma and cartilage. Three months after radical hysterectomy an inguinal node the size of an egg was removed.

Delagenière, Yves, and Beauchef, P. Tumeur mixte de l'utérus avec métastase tibio-péronière. *Ann. d'anat. path.*, 1927, 4, 617-620.

Age 54. Hemorrhages from uterus. Lobulated mass in vagina, attached by broad base within body of uterus. Histology: Cartilage, osteoid tissue. Tibia amputated 10 months after total hysterectomy for sarcoma (metastasis?). Death 14 months after hysterectomy.

Durante, G., and Roulland, H. Tumeur embryonnaire maligne de l'utérus (myxo-chondrome. *Gynécologie*, 1924, 23, 193-211. (Also: *Bull. Soc. d'obst. et de gynéc.*, 1924, 13, 28-30.)

Age 52. Metrorrhagia lasted from 4 to 6 weeks. Cervix dilated by soft malodorous masses. Abdomen tender and distended. Tumor masses sprang from corpus and cervix. Histology: Myxomatous tissue, including islands of cartilage. Death 4 months after total hysterectomy.

Fels, Erich. Misch tumor des Corpus uteri. *Monatsschr. f. Geburtsh. u. Gynäk.*, 1928, 78, 279-287.

Age 66. Vaginal discharge with slight bleeding; pains in back for 3 months. Submucous myoma covered by atypical tissue attached to anterior and right lateral wall. Histology: Islands of sarcoma, cartilage, muscle(?), and atypical epithelial cells. Six months after radical hysterectomy and irradiation, recurrence in vaginal pouch. Histology: Carcinoma, no cartilage.

Frankl, Oskar (case 1). Über Koinzidenz und Interferenz von Uterustumoren. I. Myom und Sarcom. *Arch. f. Gynäk.*, 1924, 122, 554-584.

Age 58. Almost constant vaginal bleeding, 6 months. Necrotic mass projected through external os of cervix. Entire endometrium replaced by necrotic and hemorrhagic polypoid masses. Histology: Round cell sarcoma, islands of cartilage.

Frankl, Oskar (case 2). *Ibid.*

Age 49. Metrorrhagia 4½ years. Uterus size of child's head. Histology: Spindle cells, giant cells, cartilage.

Gaebelein, M. Eine heterologe Mischgeschwulst des Uterus: Myosarcoma myxomatodes et enchondromatodes polyposum uteri. Thesis, Halle, 1909.

Age 50. Metrorrhagia, pain in lower abdomen. Polypoid mass projected through cervical os. Tumor attached to anterior and posterior walls. Histology: Cartilage, myxosarcoma, spindle cells and columnar cell epithelium, not atypical. Recurrence 4 months after radical hysterectomy.

Gebhard, C. Eine Mischgeschwulst des Uterus (Endotheliom mit Fett- und Knorpelgewebe). *Ztschr. f. Geburtsh. u. Gynäk.*, 1903, 48, 111-121.

Age 56. Watery and sanguineous vaginal discharge, cramplike abdominal pain; feeling of pressure upon bladder. Tumor, size of fist, filled upper part of vagina. Polypoid mass sprang from right tubal angle. Histology: Sarcoma, cartilage, smooth muscle, adipose tissue, glands and endothelium.

Geisler, A. Über Sarkoma uteri. Thesis, Breslau. 1891.

Age 50. Tumor attached to left lateral wall. Histology: Chondromyxosarcoma. Death 2 days postoperative.

Hartfall, Stanley J. (case 1). Chondro-sarcoma of the uterus. *J. Obst. & Gynaec. Brit. Emp.*, 1931, 38, 593-600.

Age 46. Vaginal discharge, at first serous, then serosanguineous and offensive, 18 months. Vagina filled with necrotic tissue. Polypoid tumor arose in region of right cornu. Histology: Fibrosarcoma with cartilaginous foci. Recurrence after 14 months. Death 23 months after hysterectomy. Metastases to lungs. Histology: Myxomatous tissue and cartilage.

Hartfall, Stanley J. (case 2). *Ibid.*

Age 54. Intermittent pains in lower abdomen ("like labor pains") at intervals of about 3 weeks. Uterus enlarged to that of pregnancy at 7 months. Necrotic tumor expanded uterine cavity. Histology: Partially calcified cartilage and myxomatous foci. Death 6 months after pan-hysterectomy. Recurrence in pelvis and pleura. Histology: Myxomatous tissue with islands of cartilage.

Hartfall, Stanley J. (case 3). *Ibid.*

Age 66. Irregular vaginal bleeding, at first associated with colicky pain, 12 months. Papillomatous growth on posterior wall of uterine cavity. Histology: Round and spindle cells and small giant cells; islands of cartilage. Patient apparently well for at least 5 years after vaginal hysterectomy.

Hofbauer. Rezidivierender Tumor der Corpusschleimhaut. *Monatsschr. f. Geburtsh. u. Gynäk.*, 1909, 29, 659-661.

Age 59. Vaginal discharge and occasional aching lower abdominal pain, 1 year. Lobulated polypoid mass inserted near ostium of right tube. Histology: Cartilage, sarcoma, proliferated lymphatic lining cells (probably not malignant), glands.

Jessup, D. S. Mixed tumor of the uterus. *Proc. New York Path. Soc.*, 1913, n.s. 13, 81-83.

Age 66. No clinical history. Soft, friable tissue protruded from fundus. Spindle and giant cells, islands of cartilage, alveoli and sheets of epithelium. Patient died 10 weeks after panhysterectomy following rupture of intestinal anastomosis.

Kaufmann, E. (case 1). Pathology. (English translation by Reimann, S. P.) P. Blakiston's Son & Company, Philadelphia, 1924.

No metastases at necropsy. Case of diffuse sarcoma of endometrium with cartilaginous inclusions.

Kaufmann, E. (case 2). *Ibid.*

Age 72. No clinical history. Tumor size of man's head. Histology: Fibromyoma, myxosarcoma, cartilage, bone and glands.

Kistler, Gene H. A papillary mixed tumor of the body of the uterus. *Am. J. Cancer*, 1932, 16, 399-411.

Age 64. Vaginal bleeding 3 weeks; pain in lower abdomen 1 day. Mass of opaque gray tissue projected into uterine cavity from attachment upon left and fundic walls. Histology: Adenocarcinoma, islands of cartilage. No evidence of recurrence 9 months after removal of uterus and adnexa and deep X-ray irradiation.

Kleine, H. O. Erstmalige Beobachtung eines Neuroms in der Uteruswand (kombiniert mit einer heterologen mesodermalen Mischgeschwulst). *Arch. f. Gynäk.*, 1931, 147, 680-687.

Age 44. Menorrhagia, several months. Large gray mass, occupying most of uterus, seemed to arise from myometrium of fundus. Histology: Spindle cells, myxomatous elements, cartilage. Smaller egg-sized yellow mass which did not seem to infiltrate the former. Histology: Neuroma composed of myelinated nerve fibers. No evidence of recurrence 2 years after removal of uterus and adnexa.

Köhler, R. Myxochondrosarcoma uteri. *Zentralbl. f. Gynäk.*, 1919, 43, 113-115.

Age 67. Vaginal bleeding, 2 months. Ovoid necrotic tumor mass in vagina. Numerous pillow-like masses projected from wall of uterus everywhere. Histology: Undifferentiated cells, myxomatous tissue, cartilage.

Lahm, W. Heterologe Tumorbildungen des Müllerschen Ganges im Bereich der Cervix und des Corpus uteri (Mischtumoren). In: Halban, J. and Seitz, L. Biologie und Pathologie des Weibes, IV. Urban and Schwarzenberg, Berlin, 1928, p. 652.

No clinical data. Polypoid mass attached in region of left tubal ostium. Histology: Carcinoma, myxochondrosarcoma.

Malapert, P., and Morichau-Beauchant, R. Tumeur conjonctive mixte (myxo-chondro-sarcome) de l'utérus. *Bull. et mém. Soc. anat. de Paris*, 1905, 80, 391.

Age 41. Uterine hemorrhages; colicky pain in abdomen. Uterus enlarged and vagina filled by friable mass; exact attachments not known. Histology: Spindle cell sarcoma; many nodules of cartilage. Death 13 months after onset of bleeding following total removal of the intra-vaginal mass. Local recurrence of this; uterus greatly enlarged.

McDonald, John R., Broders, Albert C., and Counseller, Virgil S. Sarcoma of the endometrial stroma. *Surg., Gynec. & Obst.*, 1940, 70, 223-229.

Age 61. No clinical data. Histology: Fibrochondrosarcoma. Lived at least 1 year.

Murray, H. Leith, and Littler, R. Meredith. A case of "mixed tumour" of the uterus (adeno-chondro-sarcoma). *J. Obst. & Gynaec. Brit. Emp.*, 1914, 25, 26-30.

Age 46. Malodorous, watery, vaginal discharge. Soft polypoid growths replacing endometrium. Histology: Glands, sarcomatous tissue, islands of cartilage.

Nicholson, G. W. Studies on tumour formation. VIII. The mixed tumours. *Guy's Hosp. Rep.*, 1924, 74, 81-108.

No clinical data. Histology: Cartilage and columnar cells.

Olinder, Ragnar. A case of malignant mixed tumour in the uterus. *Acta path. et microbiol. Scandinav.*, 1933, suppl. 16, 314-321.

Age 52. Sanguineous vaginal discharge, 5 months. Uterus hard, uneven and about size of 2 months' pregnancy. Tumor in posterior wall seemed to replace myometrium in part. Histology: Cartilage, myxomatous and "cytogenic" tissue. Local recurrence 7 months after hysterectomy. No roentgenographic evidence of pulmonary metastases at this time.

Penkert, M. Eine teratoide Mischgeschwulst des Uterus. (Carcinoma corporis uteri polyposum mit myxomatösem, sarkomatösem, und knorpeligem Stroma). *Beitr. z. Geburtsh. u. Gynäk.*, 1905, 9, 488-499.

Age 62. Sanguineous vaginal discharge; occasional pains in lower body. Large, tense mass projected above pubis. Uterus 12 by 8 by 6 cm. Polypoid tumor attached to anterior wall. Histology: Islands of cartilage, epithelial nests and cysts, giant cells, endothelium(?). No evidence of metastases at time of radical hysterectomy.

Perlstein, Isidor. The mesodermal mixed tumors of the uterus. Report of a case of botrioid chondrosarcoma of the endometrium. *Surg., Gynec. & Obst.*, 1919, 28, 43-55.

Age 54. Pain in lower abdomen and back, vaginal discharge for 1½ years. Botryoid yellow masses replaced endometrium except on anterior wall. Histology: Cartilage and stroma of round cells. Ultimately recurred.

Petersen, A. J. (case 1). Mixed tumors of the uterus. *J. Lab. & Clin. Med.*, 1922-23, 8, 369-374.

Age 60. Tumor 8 cm. in diameter; many mucosal polyps. Histology:

About 2 per cent bone, 35 per cent hyaline cartilage, 35 per cent smooth muscle, 30 per cent connective tissue, 1 per cent alveoli of round and spindle-shaped cells.

Petersen, A. J. (case 2). *Ibid.*

Age 54. Histology: One per cent cartilage and smooth muscle, 5 per cent fibrous connective tissue, 94 per cent fatty areolar tissue. Patient well 2 years after operation.

Rankin, Fred W., and Broders, Albert C. (case 1). Primary fibromyxochondrosarcoma of endometrial stroma. *Am. J. Surg.*, 1931, 12, 74-75.

Negress, aged 36. Menorrhagia and leukorrhea for 18 months. Uterus three times normal size. Originating in the posterior endometrial wall upon a broad base, was a gelatinous and cartilaginous cauliflower-like growth that slightly infiltrated the myometrium. Histology: Oval and stellate cells; fibrous and myxomatous tissue, cartilage.

Reid, W. L. Notes on a case of chondrosarcoma of the uterus. *Glasgow M. J.*, 1902, n.s. 57, 371-374.

Age 59. Copious, malodorous vaginal discharge, 4 months; sanguineous discharge, 2 months. Uterus enlarged to size of 4 months' pregnancy. Cavity filled by lobulated tumor. Histology: Myxomatous tissue; islands of cartilage.

Reinecke, Hans. Drei verschiedenartige heterologe mesodermale Kombinationsgeschwülste des Uterus. *Ztschr. f. Geburtsh. u. Gynäk.*, 1933, 104, 140-157.

Age 66. Uterine hemorrhage, 6 months. Uterus 13 by 7 by 8.5 cm. Attached by broad base in right tubal angle upon a myoma was a soft polypoid mass. Histology: Cartilage, undifferentiated sarcomatous stroma, adenocarcinoma, giant cells. Recurrence in retroperitoneum 9 months, and death 11 months, after total hysterectomy and X-ray therapy.

Ritter, Otto. Über einen mesenchymalen Misch tumor des Uteruskörpers. *Ztschr. f. Geburtsh. u. Gynäk.*, 1926, 89, 266-271.

Age 58. Uterine bleeding for several weeks. Histology of curettings: Edematous fibrillar tissue, polymorphous cell sarcoma, osteoid tissue. Uterus, removed later, 8 by 5.5 by 4 cm. Histology: Epithelium, small-celled masses resembling blastema of Wilms's tumor.

Schröder, R., and Hillejahn, A. Über einen heterologen Kombinationstumor des Uterus. *Zentralbl. f. Gynäk.*, 1920, 44, 1050-1058.

Age 58. Severe metrorrhagia, 3 weeks. Polypoid mass attached 2 cm. above internal os. Histology: Cartilage, carcinoma, fatty tissue, "perithelioma," nervous tissue. Ovary the seat of a papillary tumor. Death 13 months following radical hysterectomy and irradiation.

Seydel, Otto. Ein Enchondrom des Uterus. Ein Beitrag zur Genese der Misch tumoren des Uterus. *Ztschr. f. Geburtsh. u. Gynäk.*, 1901, 45, 237-271.

Metrorrhagia, 3 months. Tumor mass filled vagina and was attached by broad base between corpus and cervix. Cartilage with sarcomatous(?) spindle cell stroma.

Sophian, Lawrence (case 1). Adenosarcoma of body of uterus. *Am. J. Obst. & Gynec.*, 1932, 24, 911-914.

Age 55. Backache and urinary urgency. Uterus much enlarged. Dilated cavity filled by pedunculated mass. Histology: Carcinoma, myxomatous stroma, cartilage, smooth muscle. Death 14 months after removal of uterus and adnexa.

Sophian, Lawrence (case 2). *Ibid.*

Age 64. Vaginal bleeding, 4 months. Lumen filled by yellow fungating, pedunculated growth attached to fundus. Histology: Carcinoma, myxomatous stroma, cartilage, smooth muscle. No evidence of recurrence 11 months after hysterectomy and removal of adnexa.

Stout, A. P. Human Cancer. Lea & Febiger, Philadelphia, 1932.

Age 50. Menorrhagia for 30 months ending with foul discharge. Uterus the size of pregnancy at term. Histology: Huge masses of hyaline cartilage, undifferentiated cells, papillary epithelium.

van Akkeren, R. Zwei seltene Fälle von Gebärmuttergeschwulst. *Zentralbl. f. Gynäk.*, 1930, 54, 905-913.

Age 60. Scanty, odorless, vaginal discharge 5 weeks; urgency and dysuria, 4 weeks. Suprapubic mass extended to 3 cm. below the umbilicus. Friable mass filled the entire uterine cavity. Histology: Cartilage. Recurrence 3 months after hysterectomy, X-ray irradiation. Necropsy 10 months postoperative. Tumor in bones, liver and lungs. Histology: Sarcoma with giant cells, no cartilage.

Wagner, E. Verjauchende Enchondrome des Uterus, Lungenenchondrome, frische Peritonitis. Der Gebärmutterkrebs. Leipzig, 1854, p. 129. (Cited in Williams, J. Whitridge. *Am. J. Obst.*, 1894, 29, 721-764.)

Age 55. Thin-walled intra-uterine cyst from whose inner surface many cartilaginous villi projected. About fifteen nodules with similar structure in each lung. Histology: Hyaline cartilage, spindle-shaped and star-shaped elements.

Wiener, Solomon. A mixed cell tumor of the uterus. *Am. J. Obst. & Gynec.*, 1924, 8, 211-215.

Age 50. Vaginal bleeding; cramplike abdominal pain. Polypoid mass protruded into vagina through the cervical canal. Histology: Fibromyxochondroma contained islands of glandular tissue, some not of endometrial type. Hysterectomy 3 months after polypectomy. Huge polypoid mass projected into uterine cavity from all sides.

Wolfe, Samuel A. Mixed tumor of the body of the uterus. *Am. J. Obst. & Gynec.*, 1930, 19, 816-822.

Age 55. Sanguineous vaginal discharge; tender, irregular mass in abdomen extended to umbilicus. Large uterine cavity filled with tuberous mass arising from posterior, anterior and lateral walls. Histology: Cartilage, osteoid tissue, smooth muscle and spindle cells.

Mixed Tumors Containing Striated Muscle

Amolsch, A. L. Mixed mesodermal tumors of the uterus and vagina. With report of six cases. *Am. J. Cancer*, 1939, 37, 435-444.

- Age 57. Vaginal bleeding, pain in lower abdomen and slight enlargement of lower abdomen, 2 years following removal of "glandular endometrial polyp" attached high in uterus. At laparotomy, uterus greatly enlarged and miliary metastases upon peritoneum. Large polypoid mass sprang from posterolateral endometrium at cornu of uterus. Histology: "Polymorphous malignant stroma," cartilage, bone, striated muscle.
- Anderson, A., and Edmansson, Ernst. Rhabdomyoma und mehrere andere Geschwülste in einem Uterus. Nord. medic. Arkiv., Bd. 1, No. 4. (Quoted in: *Jahresb. ii. d. Leistung. in d. ges. Med.*, 1869, 4, 187-188.)
- Age 50. Vaginal discharge for several years. Vagina filled with large soft tumor that had finger-shaped extensions. Attachment at junction of cervix and corpus(?) Histology: Striated muscle, cysts lined with flattened epithelium. Death 2 months after removal of uterus.
- Bystroumow, and Eckert. Rudnew's Journal, 1874, p. 442. (Cited in Kolesnikow, N. Pigmentirtes Rhabdomyom (Rhabdomyoma melanodes). *Virchows Arch. f. path. Anat.*, 1876, 68, 554-575.)
- No clinical data. Pedunculated tumor attached to endometrium. Histology: Striated muscle, binucleate or trinucleate spindle-shaped cells.
- Colomiatti, V. Contribuzione allo studio dei tumori dell' utero. *Arch. per le sc. med.*, 1881, 5, 1-23. (Quoted by Robertson.)
- No clinical data. Tumor, size of fetal head, probably arose from the corpus. Histology: Large round cells, striated muscle.
- Frank, R. T. Gynecological & Obstetrical Pathology. D. Appleton & Company, New York, 1922.
- Age 70. No clinical history. Histology: Carcinoma, squamous epithelium, embryonal striated muscle.
- Frankl, Oskar (case 3). Über Koinzidenz und Interferenz von Uterustumoren. I. Myom und Sarkom. *Arch. f. Gynäk.*, 1924, 122, 554-584.
- Age 47. Menorrhagia and dysuria. Necrotic and hemorrhagic cauliflower-like mass sprang from posterior wall of uterus. Histology: Striated muscle, cartilage, osteoid tissue, "atypical stroma."
- Gamper, Alfred. Beitrag zur Kenntniss der mesodermalen Mischgeschwülste des Uterus. *Arch. f. Gynäk.*, 1926-27, 129, 878-890.
- Age 54. Yellow vaginal discharge for 16 weeks, blood stained for 4 weeks. Soft friable mass protruded from external os of cervix. Uterus 10 by 8 by 7 cm. Endometrium was the source of masses arising everywhere but from anterior wall. Histology: Rhabdomyosarcoma, cartilage, occasional glands, spindle cell stroma. Patient in good health 4 years after hysterectomy.
- Glynn, Ernest, and Bell, W. Blair (case 1). Rhabdomyosarcoma of the uterus. *J. Obst. & Gynaec. Brit. Emp.*, 1914, 25, 1-12.
- Age 62. Bloody vaginal discharge for 4 months. Passage of mass the size of an orange *per vaginam*. Ovary the seat of a columnar cell carcinoma. Uterus the size of a 3 months' pregnancy. Polypoid tumor arose from posterior wall of uterus. Histology: Rhabdomyosarcoma with stroma of oval or spindle-shaped cells. Six months later pulmonary symptoms suggested metastases.

Glynn, Ernest, and Bell, W. Blair (case 2). *Ibid.*

Age 75(?). Scanty blood-tinged vaginal discharge (3 months); passed large mass *per vaginam*. Polypoid growths attached to posterior wall. Histology: Striated muscle cells, small round and spindle-shaped cells, multinucleated cells. Recurrence in abdomen 2 months after radical hysterectomy. Histology: Myxosarcoma.

Gunning, R. E. Lee, and Ross, Charles A. Rhabdomyosarcoma of the corpus uteri. *Surg., Gynec. & Obst.*, 1940, 70, 230-233.

Age 58. Passage of blood clots and foul-smelling discharge *per vaginam*. External os full of necrotic tissue and blood. Nodular gray gelatinous mass originated from the posterior and lateral walls. Histology: Almost all cells of the tumor show cross striations.

Halter, Gustav. Heterotoper Misch tumor des Corpus uteri. *Zentralbl. f. Gynäk.*, 1926, 50, 2194-2196.

Age 41. Menorrhagia for 12 years. Ten months before examination had a severe hemorrhage followed by passage of "myoma" *per vaginam*. Vagina filled with necrotic, polypoid masses. Uterus size of 5 months' pregnancy. Polypoid mass attached by broad base to posterior wall of corpus. Histology: Striated muscle, glands lined by simple cuboidal epithelium (did not look like carcinoma), embryonal connective tissue. Patient well 3 months following total hysterectomy.

Herb, Isabella C. Mixed tumors of the uterine body. *Surg., Gynec. & Obst.*, 1910, 10, 463-467. (Also, more briefly, in *Tr. Chicago Path. Soc.*, 1909-12, 8, 5-7.)

Age 55. Pelvic discomfort for 8 months; vaginal discharge, at times hemorrhagic. Uterus 10 by 7.5 by 6.5 cm. Firm mass, filling entire uterine cavity and penetrating the wall, arose from fundus and region of the right fallopian tube. Histology: Smooth and striated muscle, multinucleated cells, cylindrical cells and round and polygonal cells. Local recurrence with death 6 months after supracervical hysterectomy and right salpingo-oophorectomy.

Hunziker, Hans. Die Rhabdomyome des Corpus uteri. *Beitr. z. Geburtsh. u. Gynäk.*, 1908, 12, 317-337.

Age 58. Painless vaginal discharge, 5 to 6 weeks. Pelvic mass extended 1 to 2 cm. above symphysis pubis. Mass 6 by 4 by 5 cm., attached to anterior endometrium near ostium of left tube, dilated the uterine cavity. Histology: Round and spindle cell stroma, striated muscle, epithelium that did not have a malignant appearance. Death 5 months after hysterectomy with recurrence in pelvis. Histology: Striated muscle, no cartilage.

Läwen, A. Über ein Rhabdomyosarkom des Uterus mit drüsigen Wucherungen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1905, 38, 177-206.

Age 60. Slight vaginal bleeding. Uterus size of child's head. Polypoid masses, arising from fundus, posterior wall and ostium of right fallopian tube, involved also the cervix. Histology: Adenocarcinoma, spindle cell sarcoma, striated muscle. Death 1½ years after panhysterectomy, with evidence of peritoneal metastases and peritonitis.

Lochrane, C. D. Rhabdomyosarcoma of corpus uteri. *Proc. Roy. Soc. Med.*, 1933, 26, 1429-1435.

Age 56. Exsanguination from vaginal hemorrhages of few weeks' duration. Firm friable mass protruded through dilated cervical canal. Uterus size of 10 months' pregnancy. Mass attached to anterior wall of uterus just above internal os. This was locally removed; then hysterosalpingo-oophorectomy. Histology: Rhabdomyosarcoma.

Reeb, and Oberling, Ch. Rhabdomyosarcome et épithélioma cylindrique du corps utérin. (Dysembryome de l'ovaire droit). *Gynéc. et obst.*, 1929, 19, 81-90.

Age 51. Profuse uterine bleeding and foul discharge for 1 month. Uterus size of 8 months' pregnancy. Soft masses filled vagina. Polypoid mass sprang from fundus. Histology: Giant cells, striated muscle cells, adenocarcinoma, undifferentiated sarcoma. Metastasis in peritoneum. Histology: Rhabdomyosarcoma. Dermoid cyst in right ovary. Total hysterectomy. Vaginal recurrences 2 months postoperative and death several weeks later.

Robertson, A. Rocke. Rhabdomyosarcoma of the uterus; with the report of a case. *J. M. Research*, 1909, 20, 297-309.

Age 69. Slight bloody vaginal discharge for 6 months. Red, hard, immovable mass filled upper part of vagina. Source of this was lower portion of corpus and cervix. Histology: Large multinucleated cells; striated muscle cells. No evidence of metastases at necropsy 2 months later.

Shapiro, Phillip F. Rhabdomyosarcoma of the corpus uteri. *Am. J. Obst. & Gynec.*, 1931, 21, 83-91.

Age 52. Dull pains in lower abdomen for 2 months; nausea, weakness, loss of weight and fever for 2 weeks. Death 24 hours after admission. Uterus enlarged to 18 by 14 by 8.5 cm. by submucosal myoma and by a boggier subserous mass between this and the left ovary. Histology: Rhabdomyosarcoma. No metastases at necropsy.

von Franqué, Otto. Ueber Sarcoma uteri. *Ztschr. f. Geburtsh. u. Gynäk.*, 1899, 40, 183-243.

Age 49. Irregular menorrhagia for 4 years. Increase in size of abdomen. Abdominal pain and foul-smelling vaginal discharge, 2 weeks. Abdominal mass the size of man's head; soft tumor masses palpable through patulous cervical canal. Soft mass arose from posterior and left walls of uterus. Histology: Round and spindle-shaped cells; smooth and striated muscle fibers. No evidence of metastases at necropsy 1 week after total abdominal hysterectomy.

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- Manheims, Perry J. Carcino-sarcoma of the uterus. *Proc. New York Path. Soc.*, 1923, n.s. 23, 74-78.
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- thelwucherungen im Uterus des Kaninchens mit Knorpel- und Knochenbefunden. *Ztschr. f. Krebsforsch.*, 1938, 47, 1-12.
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DESCRIPTION OF PLATES

PLATE I

- FIG. 1. Case I. Gross specimen in sagittal section. Almost actual size.
- FIG. 2. Case I. Cartilage and spindle-shaped cells. $\times 125$.
- FIG. 3. Case I. Cartilage and osteoid tissue. $\times 125$.
- FIG. 4. Case I. Tumor in vascular space. $\times 125$.

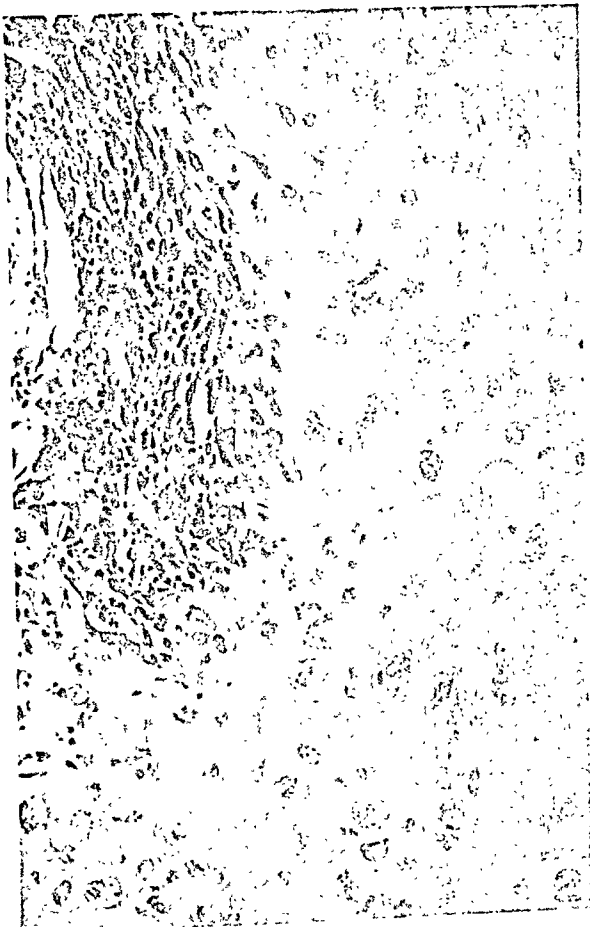
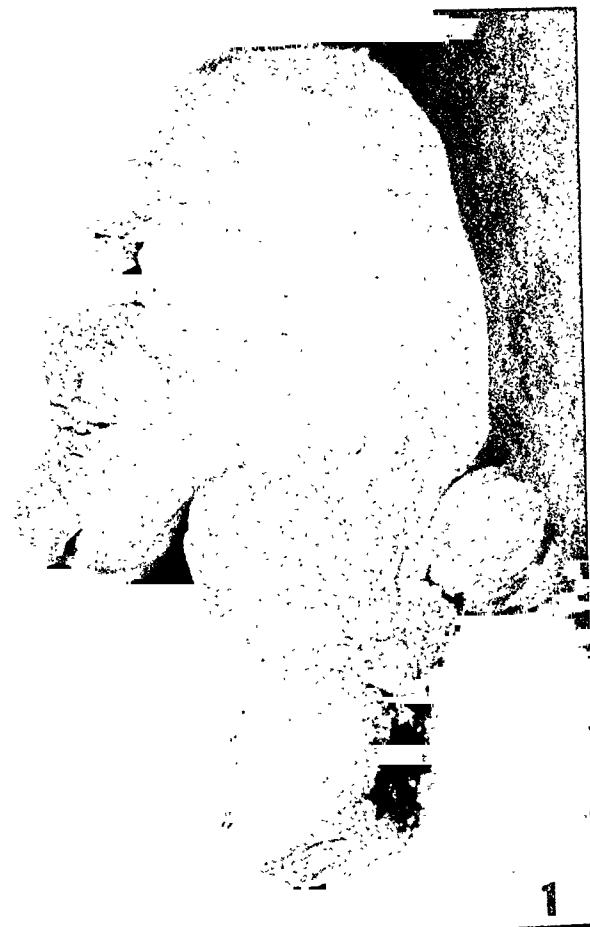


PLATE 2

FIG. 5. Human fetal cartilage from head of femur. $\times 95$.

FIG. 6. Higher magnification of a portion of the same culture as shown in Figure 5. $\times 300$.

FIG. 7. Case I. Explant from cartilaginous mass. $\times 95$.

FIG. 8. Case I. Higher magnification of a portion of the same culture as shown in Figure 7. $\times 300$.

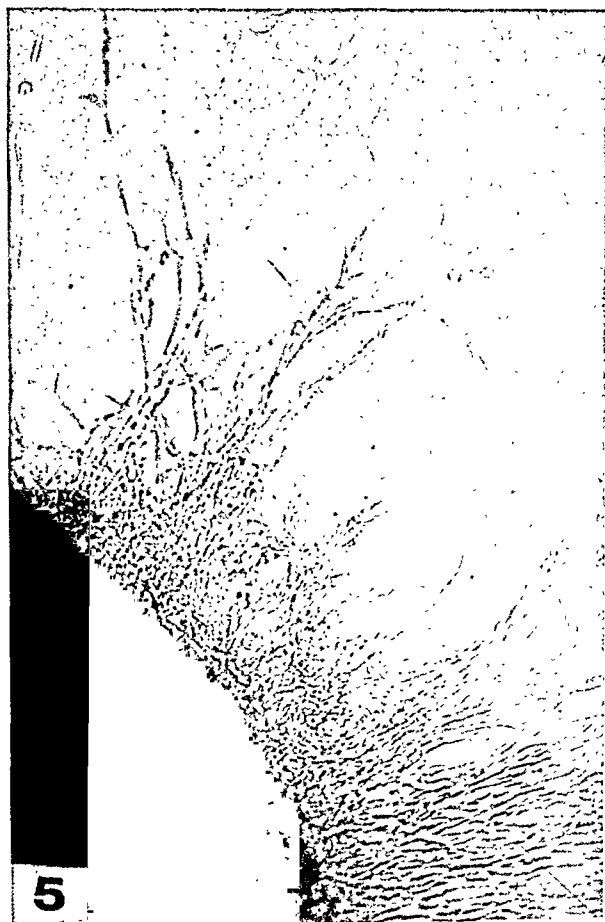
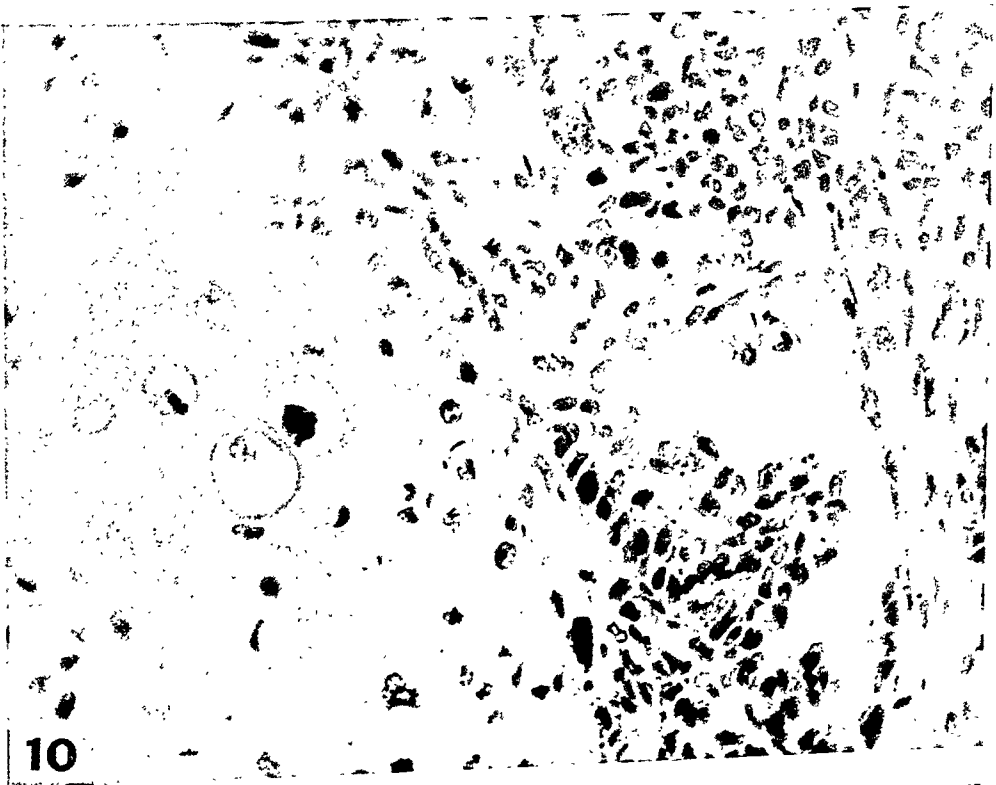
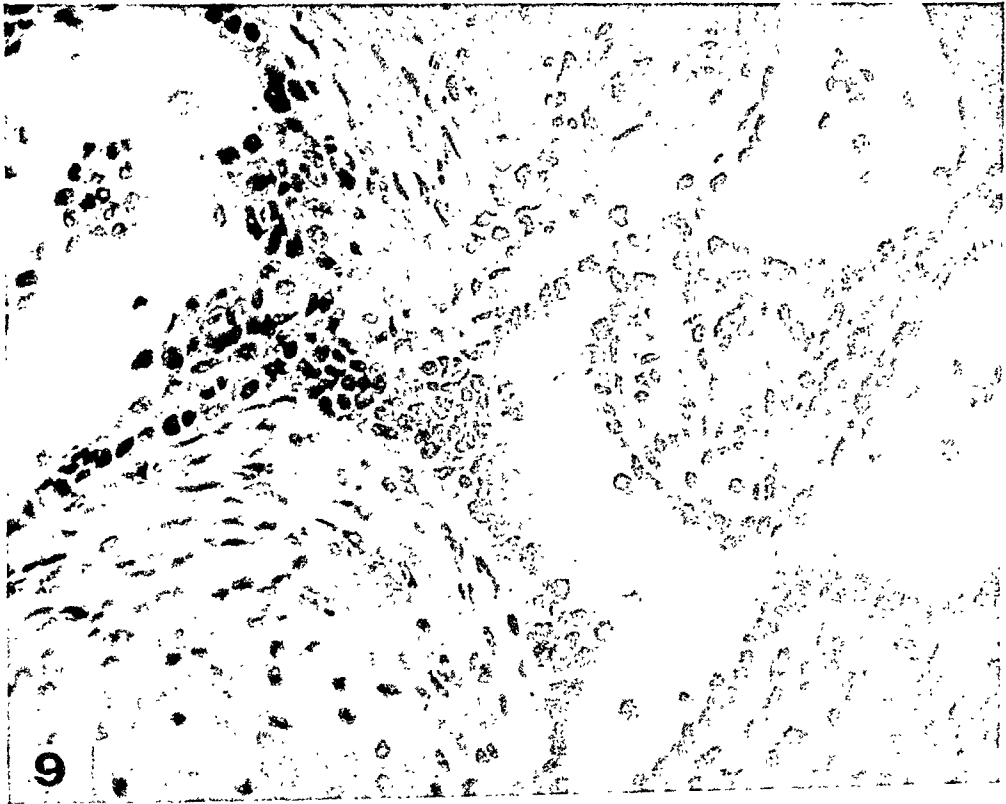


PLATE 3

FIG. 9. Case II. Mixed tissues at junctional zone. $\times 235$.

FIG. 10. Case II. Epithelium, sarcomatous stroma and cartilage. $\times 235$.



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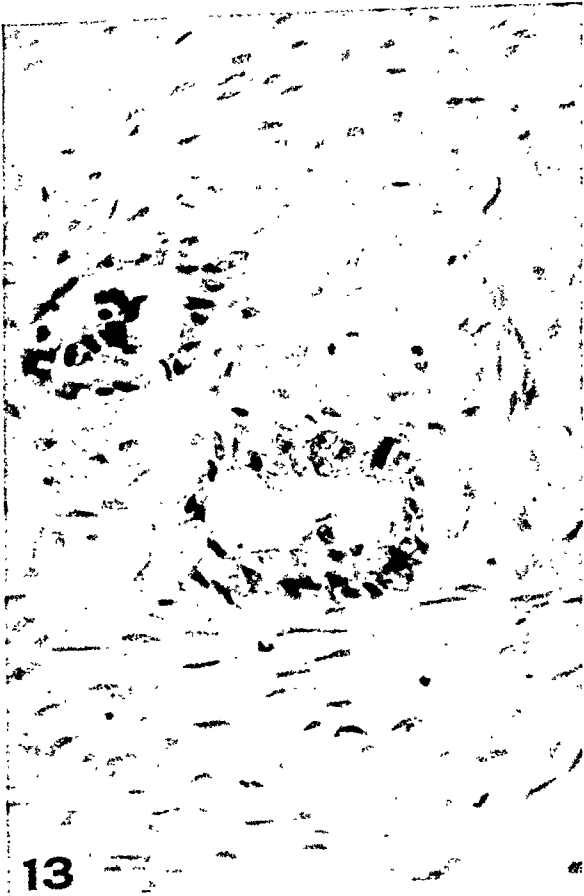
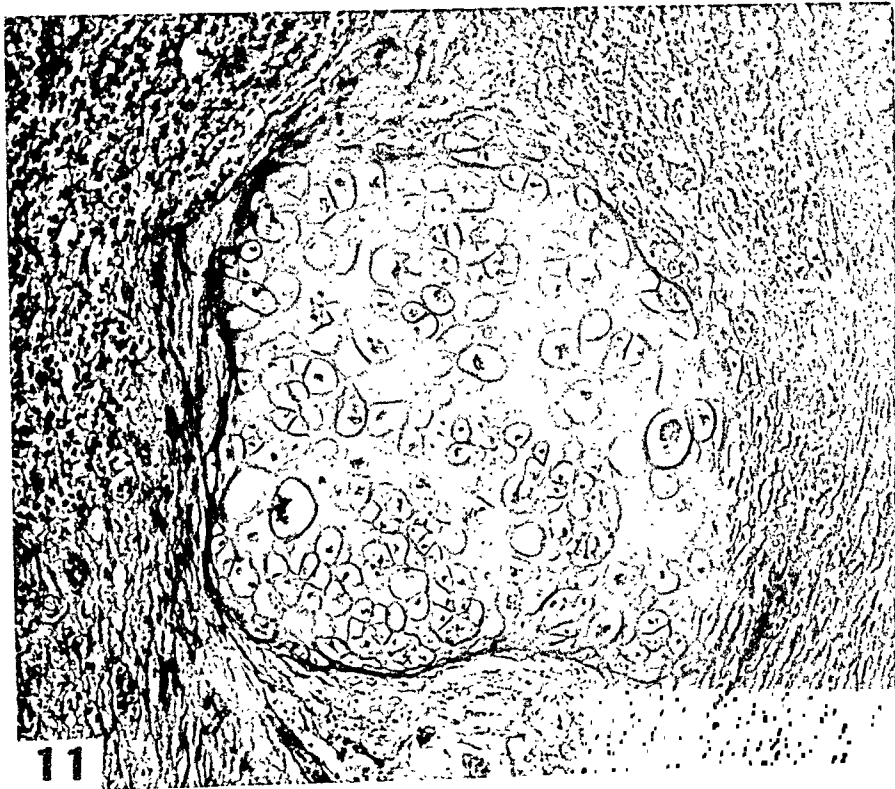
Mesodermal Mixed Tumors of the Uterus

PLATE 4

FIG. 11. Case II. Relation of reticulum to cartilage as shown in a preparation stained by the Wilder method. $\times 100$.

FIG. 12. Case II. Basement membranes of acini as shown in a preparation stained by the Wilder method. $\times 235$.

FIG. 13. Case II. Metastasis in omentum; adenocarcinoma in myxomatous stroma. $\times 235$.



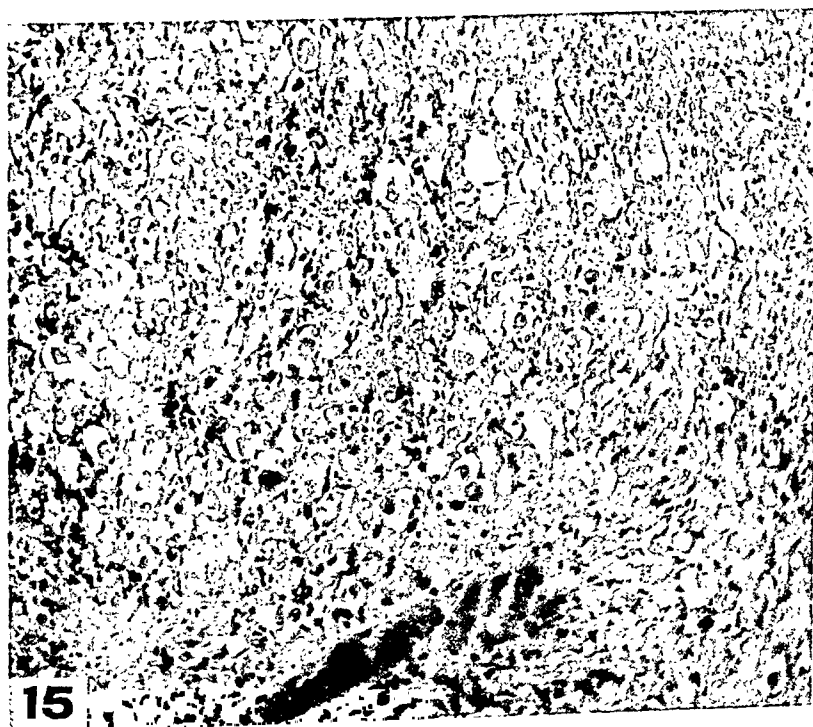
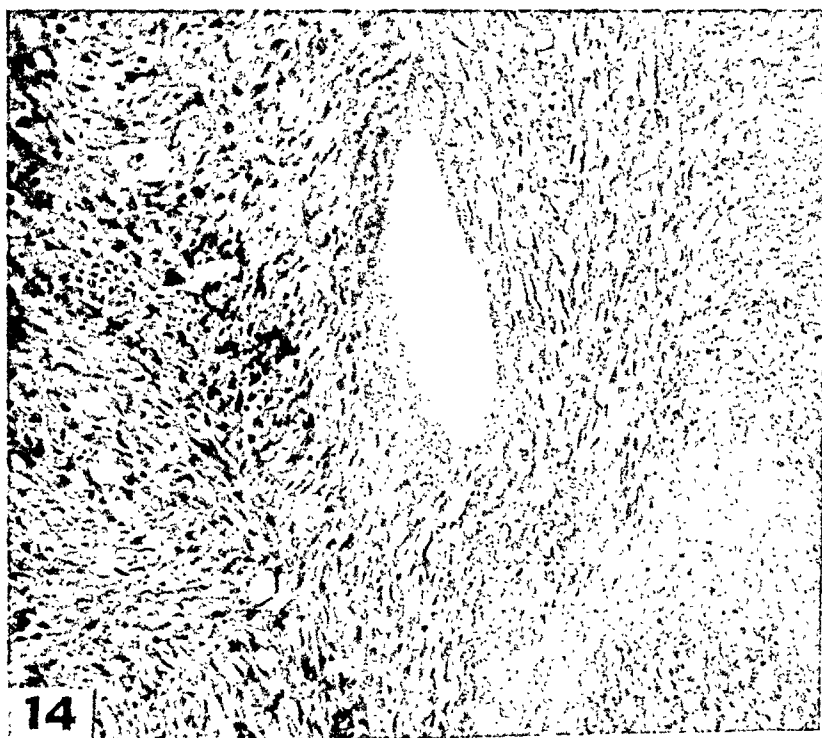
Mesodermal Mixed Tumors of the Uterus

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PLATE 5

FIG. 14. Case III. Acinus and spindle-shaped cells. Foci of necrosis and hemorrhage. $\times 125$.

FIG. 15. Case III. Giant cells. $\times 125$.

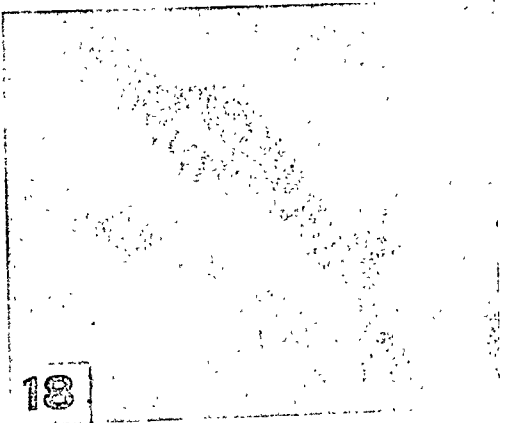


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Mesodermal Mixed Tumors of the Uterus

PLATE 6

FIGS. 16, 17, 18, 19, 20. Case III. Striated muscle fibers. $\times 1000$.



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Mesodermal Mixed Tumors of the Uterus

EXPERIMENTAL HYPERTENSION AND PREGNANCY IN DOGS*

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Goldblatt, Lynch, Hanzal and Summerville¹ found that constriction of the renal arteries may result in a sustained elevation of the blood pressure. Dill and Erickson² stated that the production of renal ischemia in pregnant dogs or rabbits resulted in an eclampsia-like syndrome, characterized by a rapidly fatal course and significant pathological lesions in the kidneys and liver. In earlier unreported experiments, Mason, Harrison and Blalock noted that pregnant dogs with a preëxisting hypertension due to renal ischemia had a temporary decline in blood pressure without evidence of uremia or eclampsia at the terminal part of the pregnancy period. Similar observations have been made by Goldblatt on dogs and by Williams and Harrison on rats.

METHODS AND RESULTS

The method of Goldblatt and co-workers¹ for producing hypertension was employed, the blood pressure being determined by the direct needle puncture method.

Thirty-one animals were used in this study. Constriction of the renal arteries was produced in 26; 1 animal had a spontaneous hypertension, 2 animals aborted without renal artery constriction having been produced, and hepatic artery occlusion was carried out on the 2 remaining animals. Of the 26 animals with the constriction of renal arteries, abortion or resorption of fetuses occurred in 15, 9 gave birth to live puppies, and 2 died from uremia without either abortion or delivery having occurred.

Of the 15 animals in which abortion or resorption of fetuses occurred, 8 were killed about 24 hours after vaginal discharge was

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noted in order to obtain tissues for examination. All of these had presented at least a moderate elevation of blood pressure which in most instances declined slightly at the time of abortion. None of them had convulsive-like movements and all appeared to be in good condition. Of the remaining 7 animals in this group, 4 died after intervals varying from 4 to 13 days following constriction of renal arteries and from 3 to 14 days following abortion. The duration of the pregnancy at the time the clamps were applied was 3, 6, 7 and 7 weeks. A definite elevation in pressure followed constriction of the renal arteries. Death was due to uremia, all having a definite elevation of nonprotein nitrogen. The remaining 3 animals survived following abortion which occurred in 3 days, 3 weeks and 3 weeks following the constriction of the arteries. The duration of pregnancy at the time of application of the clamps was 1, 2 and 4 weeks respectively. All showed a moderate elevation in blood pressure which declined slightly at the time of abortion and rose slightly subsequently. These animals did not appear ill at any time.

Postmortem examinations were performed on the 8 dogs which were killed following abortion and on the 4 which died after abortion. These examinations revealed changes which corresponded to the acute lesions which have been described in nonpregnant animals following constriction of renal arteries; the necrotizing arteriolitis was widespread and it was associated with multiple infarcts of various organs in many of the dogs. In addition to these lesions which are commonly found in acute experimental hypertension, hepatic lesions were present in 4 dogs (3 of 8 which were killed and 1 of those which died). These lesions were not visible grossly and except for slight fatty metamorphosis and some congestion the livers of all dogs appeared normal. Microscopic study revealed coagulation necrosis of liver cells in the periphery of the lobules associated with polymorphonuclear and mononuclear exudate and with fibrin deposition and capillary thrombosis. The process was never associated with hemorrhage. These areas of necrosis were associated invariably with definite inflammatory changes in the stroma of the triads and occasionally there was thrombosis of an arteriole; necrosis of arterioles was not observed and thrombosis of these vessels was uncommon.

The striking feature of the microscopic lesion was the inflamma-

tory reaction in the stroma of the triad. This inflammatory reaction was both acute and chronic and in some instances was present without any demonstrable necrosis of the hepatic parenchyma. Frequently triads were seen in which the reaction was so massive and so acute that the lesion resembled an abscess. The bile ducts themselves contained no exudate and there was no necrosis of biliary epithelium. Parasites were not demonstrable in histological preparations.

Two dogs died of uremia, before either abortion or delivery had occurred, 3 and 5 days following constriction of the renal artery. There was marked elevation of nonprotein nitrogen in both. One of the animals had a moderate elevation of blood pressure and convulsions. Postmortem examination was performed on both dogs but autolysis had rendered the tissues of one useless; in the other no liver lesions were found.

Nine of the 26 dogs gave birth to normal puppies. One died 7 days following delivery and another on the 13th day. The clamps had been applied only 2 and 3 days respectively prior to delivery in these 2 animals. The liver lesions which have been described were not observed in either of these. Of the remaining 7 animals, the clamps were applied at periods ranging from 1 to 7 weeks following the onset of pregnancy in 6 and hypertension had been induced previously in 1. Elevations of blood pressure ranging from moderate to severe were observed. The common finding was an elevation following the application of clamps which declined somewhat at the time of delivery and then rose subsequently. An exception was noted in the dog with persisting induced hypertension, in which the decline in pressure did not take place at the time of delivery but occurred subsequently when it was nursing. When the pups were weaned, the pressure rose again. The response in blood pressure of an animal in which the predelivery rise in pressure was not so great as usual and the postdelivery pressure greater than usual is shown in Chart 1. These dogs did not present symptoms of renal insufficiency or eclampsia at any time.

As stated previously, one animal had a definite elevation in blood pressure (mean pressure 180 mm. Hg.) at the time it became pregnant. The pressure continued to remain elevated and Goldblatt clamps were not applied to the renal arteries. Abortion occurred 5 weeks after the beginning of pregnancy and the blood

pressure at this time declined to 130 mm. of Hg. The dog did not appear to be ill at the time it was killed. Microscopic study of the liver revealed periportal necrosis similar to that already described.

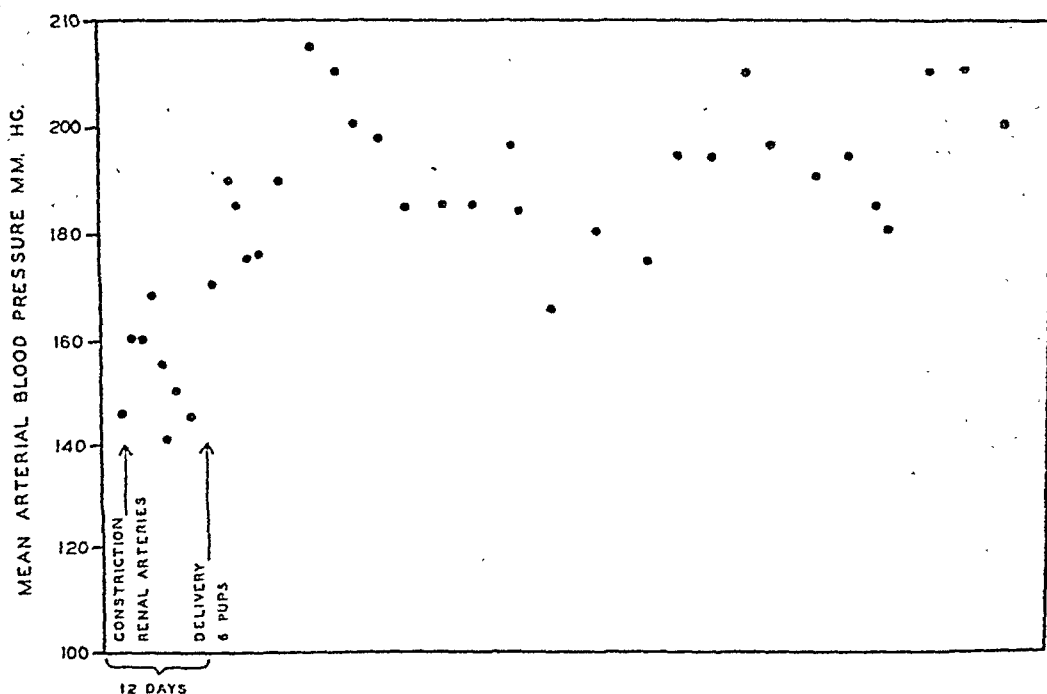


CHART I. The mean arterial blood pressure before and after delivery in a dog with renal ischemia.

The kidneys of this dog were of considerable interest and because of the infrequent occurrence of natural hypertension in dogs they will be described briefly. Grossly they were approximately normal in size, but they were pale and firm. On superficial examination numerous small clear cysts 1 to 3 mm. in diameter were seen within the capsule. The cortex was slightly narrowed and it was pale and yellowish. The striations were not distinct and they were distorted by irregular streaks or splotches of brownish tissue. The pyramids and the pelves appeared unchanged. The capsule was thickened and because of adhesions between it and the cortex it was stripped away with difficulty. The cysts were seen within the capsule and they overlay small depressions in the cortex. The blood vessels were not thickened.

Microscopic sections showed cystic spaces within the thickened capsule. These spaces were lined by very flat cells and they contained precipitated protein. Surrounding them were accumula-

tions of lymphocytes and plasma cells. This inflammatory reaction in the capsule was continuous with a similar scarring and inflammatory reaction in the cortex. The glomeruli outside of the scarred areas showed syncytial masses of cells with pinkish cytoplasm and relatively large oval nuclei. Most of these masses were near the hilum but some extended far into the glomerulus. These bodies somewhat resembled those described by Goormaghtigh³ but we have not studied them sufficiently to justify any interpretation of their significance.

Two pregnant animals with normal blood pressure aborted at 5 weeks without constriction of the renal arteries having been induced. These animals did not appear ill at the time they were killed. Examination of one of these dogs revealed no liver lesions. The tissues from the other dog were lost.

For the purpose of comparison the hepatic arteries of two pregnant dogs were occluded. This was done in stages by the method described by Huggins and Post.⁴ One of these animals aborted 5 days following the second operative procedure, which was during the fifth week of pregnancy. Liver lesions similar to those already described were found. Normal delivery occurred in the other and no liver lesions were found.

DISCUSSION

One of the most striking features of the experimental disease was that animals which appeared to be ill prior to abortion or delivery did not seem to improve subsequently. Most of these animals died and the evidence indicates that the cause of death was uremia. On the other hand, many of the animals which aborted did not appear to be ill at any time and there was little if any elevation of the nonprotein nitrogen of these animals. The cause for the abortion is not apparent. The incidence of abortion in nonhypertensive pregnant dogs in our laboratory is not known, but several of these animals aborted before the renal arteries were constricted. It is our impression that the incidence is higher than normal in the animals with renal ischemia.

In attempting to explain the contradictory results of Dill and Erickson² and of our experiments, it is possible that the former authors, in attempting to cause a marked elevation in blood pressure, produced too severe constriction of the renal arteries. Their

experiments were performed on animals in the later stages of pregnancy and, as has been stated, the blood pressure of a dog with hypertension will decline shortly before and at the time of delivery. Therefore, in order to produce a given rise in blood pressure, it is likely that the renal arterial constriction must be greater in dogs during the terminal stages of pregnancy than in normal nonpregnant animals. At any rate, it is our impression that uremia was the cause of death in most instances.

Dill and Erickson² have pointed out certain similarities between the hepatic lesions in hypertensive pregnant dogs and those in human eclampsia. In our studies hepatic lesions were also found, but we feel that these lesions bear only a superficial resemblance to those in human eclampsia. No gross hemorrhages were observed in the livers of dogs in our experiments and hemorrhage was not a feature of the microscopic lesions. The necrosis which occurred in our dogs was of the coagulation type and it was associated with fibrin deposition and fibrin thrombi in the capillaries. Polymorphonuclear leukocytes constituted the greater part of the exudate but some mononuclear phagocytes were also present. These necrotic areas were invariably adjacent to triads and the inflammatory reaction was present in the stroma of the triad as well as in hepatic parenchyma. In addition to acute inflammatory exudate, plasma cells and lymphocytes were also present in the stroma. In most, if not all instances, the lesions in the triad stroma were more conspicuous and older than the lesions in the parenchyma. No inflammatory exudate was seen in the bile ducts and the radicals of the hepatic artery and portal vein also appeared normal. Arteriolar necrosis was not a feature of the lesion.

The genesis of these lesions is unknown but it seems likely that it is different from that in eclampsia because of the absence of hemorrhage and the presence of inflammatory changes in the stroma of the portal triads. Regardless of the differences between these hepatic lesions and those which occur in human eclampsia, our experiments show that hypertension is not a necessary factor in their pathogenesis. This is shown by the occurrence of these hepatic lesions in a dog in which constriction of the hepatic artery had been produced and in which there was no elevation of blood pressure.

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DESCRIPTION OF PLATES

PLATE 7

- FIG. 1. Hepatic lesion showing necrosis of liver cells with thrombi in capillaries, and cellular infiltration of the stroma of the triad. Hematoxylin and eosin. $\times 275$.
- FIG. 2. Larger lesion showing more extensive cellular infiltration of triad. Necrosis of parenchyma is evident in the lower segment. Hematoxylin and eosin. $\times 275$.
- FIG. 3. Still larger lesion in which the cellular infiltration of the triad stroma overshadows the parenchymal lesion. Hematoxylin and eosin. $\times 200$.

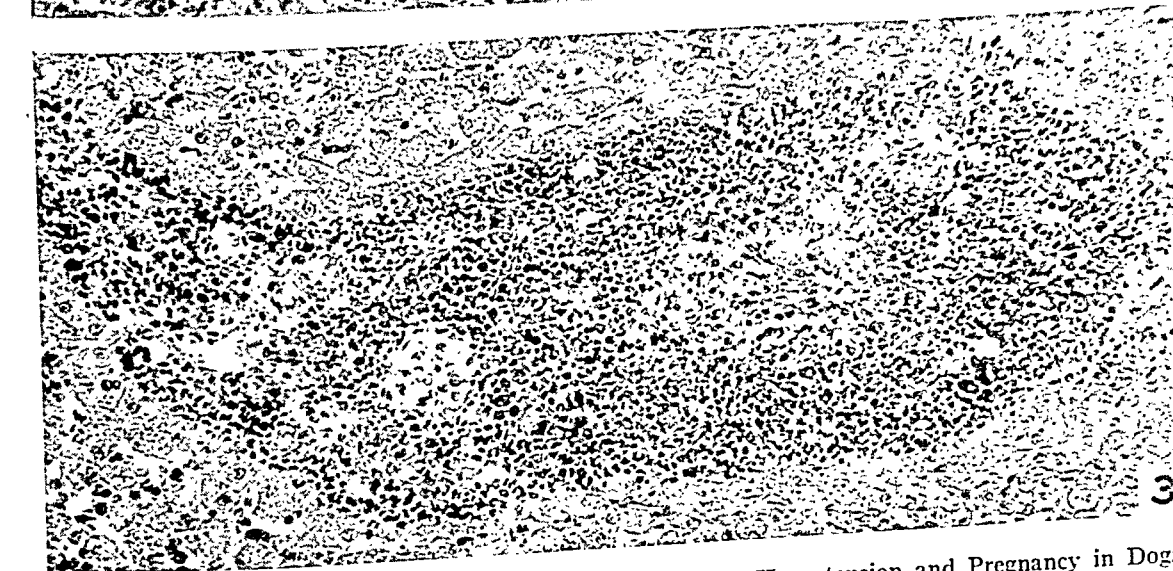
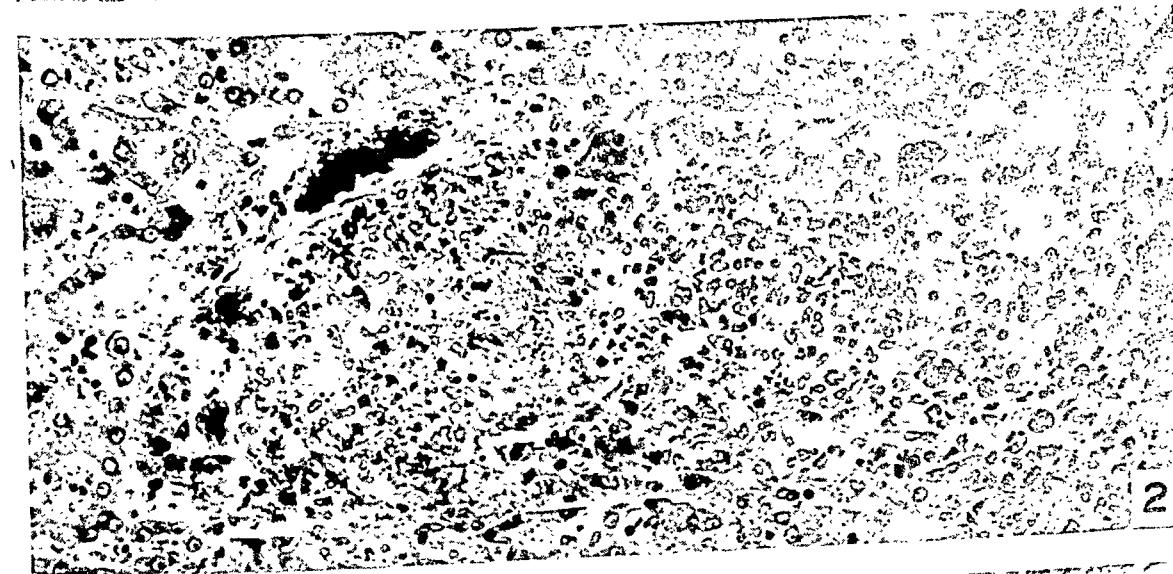
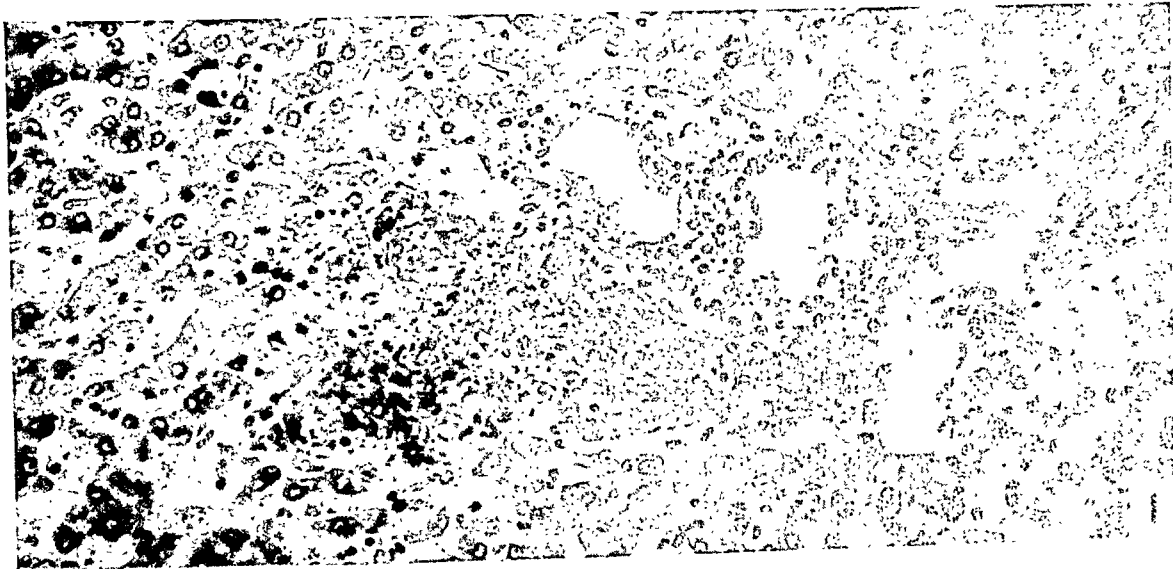
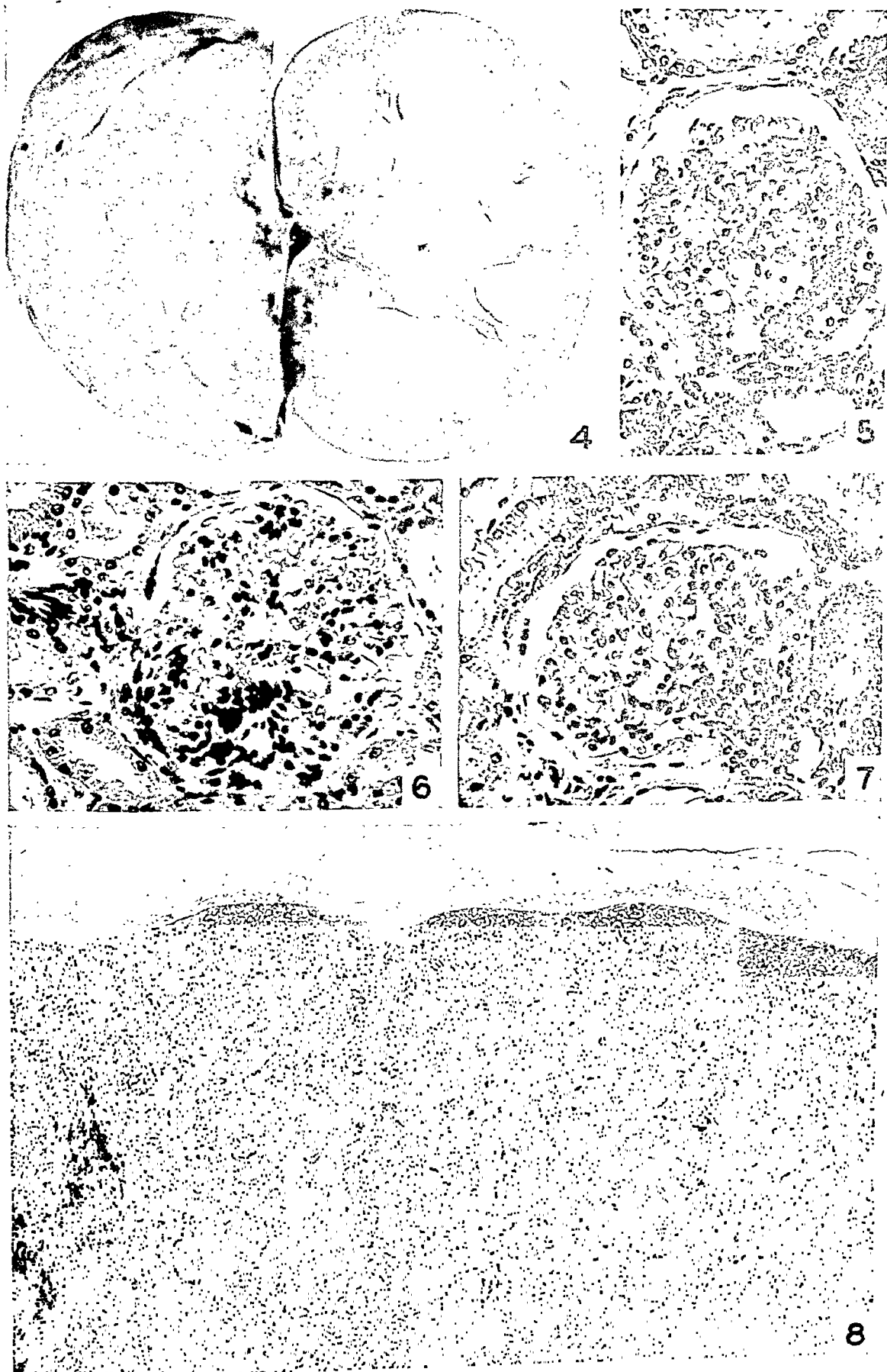


PLATE 8

- FIG. 4. The kidney of a dog exhibiting a spontaneous hypertension. Note the cysts in the capsule, narrowing of the cortex, and the irregular scars within it.
- FIG. 5. Glomerulus from this dog showing the extension of the syncytial mass far out into the glomerular tuft. Hematoxylin and eosin. $\times 225$.
- FIG. 6. Similar lesion located at hilum and extending into the glomerulus for a short distance. Hematoxylin and eosin. $\times 225$.
- FIG. 7. Lesion like that shown in Figure 6 except that it is confined almost entirely to hilum. Hematoxylin and eosin. $\times 225$.
- FIG. 8. Low power photomicrograph of kidney cortex of same dog. Note capsular cysts and inflammation of cortical scars. The syncytial masses in some glomeruli may be seen even at this low magnification. Hematoxylin and eosin. $\times 14$.



STRUCTURE OF THE SMALL CEREBRAL ARTERIES IN HYPERTENSION*

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In a previous publication,¹ a study was made of the normal histology of the small cerebral arteries and of the changes that occur in their walls with advancing age. With these observations available for comparison it seemed of interest to study the vascular alterations resulting from hypertension. In reviewing the literature on the structure of small cerebral arteries in hypertensive persons, one is impressed by the paucity of reports. There is a definite lack of agreement as to the exact character of the changes.

Johnson² and Ewald³ described a medial hypertrophy occurring within the arterioles throughout the body. These investigators, however, made no special reference to the cerebral vessels.

Jores⁴ recorded a widespread intimal hyalinization occurring within most of the arterioles, excluding those within the skeletal muscles.

Keith, Wagener and Kernohan⁵ studied the arterioles in four cases of malignant hypertension. They described a marked intimal hyperplasia with hypertrophy of the internal elastic lamina and of the media. There was some perivascular fibrosis.

Rosenberg⁶ reported changes in the brain in seventeen patients suffering from malignant hypertension. He found a thickening of the walls of the arterioles with an associated reduction in the caliber of the lumens. Intimal proliferation occurred but was not constant. The elastica interna was frequently hypertrophied, frayed and reduplicated. The media was increased in thickness.

Moritz and Oldt⁷ found the small cerebral arteries altered in but 8 per cent of their cases of hypertension. The changes consisted primarily of a medial hypertrophy and of an endothelial hyperplasia with an increase in the elastic elements.

With these investigations in mind, 53 cases of severe hyperten-

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sion were selected from our autopsy service for study. The diagnosis was substantiated by the blood pressure record, the heart weight, or both. The age groups of these individuals were as follows: 5 cases from 11 to 30 years of age; 5 cases from 31 to 40 years; 13 cases from 41 to 50 years; 17 cases from 51 to 60 years; 4 cases from 61 to 70 years; and 9 cases over 70 years of age. No attempt was made to differentiate between the malignant and the slowly progressive nonmalignant type of hypertension, as was done by Rosenberg.⁶ Most of the cases, however, were probably not examples of malignant hypertension, since 31 of the 53 had symptoms lasting more than 2 years prior to death; in 16 the symptoms had been present for 5 years or more. This is in direct contrast to Rosenberg's cases in which the average duration of life after the onset of symptoms varied from 4 to 18 months.

Blocks of tissue were taken from various regions of the brain. These blocks were selected from apparently uninvolved areas. The sections were stained with hematoxylin and eosin, with Weigert's elastic tissue stain, with the Mallory-Heidenhain technic (azocarmine) and with the Bodian stain. As in our previous study,¹ the Mallory-Heidenhain technic has proved invaluable for the study of muscle and fibrous tissue because it demonstrates even the smallest amount of these elements.

Since the structure of the normal small cerebral arteries differs from that of similar-sized vessels elsewhere in the body, it might be of advantage to review their structure briefly at this time. Only arteries varying in size from 50 to 150 μ in diameter will be considered. The internal elastic lamina is both relatively and absolutely thicker than in similar vessels elsewhere in the body. This relative thickness of the elastica tends to persist even into the smallest arteries. The media is composed primarily of a foundation of radially arranged collagenous fibers. This collagenous tissue comprises a surprisingly large portion of this layer and in many cases makes up the major part of the vessel wall. Throughout this connective tissue there are found, in varying numbers, obliquely arranged muscle cells. As the vessel decreases in size, the muscle tissue rapidly disappears and is often difficult to find in vessels under 70 μ in diameter. With increasing age, there results a progressive reduction in the quantity of the elastic and muscular elements of the media. This change is conspicuous and is nearly

complete early in life. When fibrosis is complete it is usually impossible to differentiate the media from the adventitia, the two merging to form a single structure. The adventitial layer of the small cerebral arteries is variable in size. In some cases it is composed only of a few strands of tissue, while in others it is equal in thickness to the adjoining media. As a rule it is composed of a loose network of collagenous fibers.

The cases in the present study were divided into those under 40 years of age and those over this age limit. This particular differential point was chosen because normally only a minimal degree of vascular alteration occurs prior to this age. One can detect normally some medial fibrosis, but most of the intimal and medial changes occur in individuals past the third decade of life.

AGE GROUP 11 TO 40 YEARS

Material from 10 hypertensive patients in the age group 11 to 40 years was studied. In 3, the small cerebral arteries showed extensive changes, while in the remaining 7 they presented little, if any, alteration. A review of the history of the 3 cases showing arterial change revealed the course of the hypertension to have been very rapid. They probably belong to that group described by Keith, Wagener and Kernohan,⁵ and Rosenberg⁶ as malignant hypertension. Since the alterations of the elements of the vessel wall in these cases were very pronounced, they warrant some detailed description.

Intima. Many vessels showed a marked endothelial proliferation even to the extent of complete vascular occlusion. The usually solid, thick, elastic lamina was irregularly frayed with many tiny fibrils projecting from various portions of the membrane into the adjacent media. Reduplication of the elastica, which does not normally occur until the fifth and sixth decades, was already very extensive. The reduplicated elastic elements occasionally extended inward to narrow or occlude the vessel lumen. Certain segments of the elastica interna showed a definite thickening; other segments were swollen and had lost much of their normal tinctorial properties. These swollen areas occasionally projected inward, producing a definite narrowing of the lumen.

Media. There was a partial to complete reduction of the elastic and muscular elements within the media. In those arteries where

the replacement of the muscle had been complete the fibrosed media merged with the adjacent adventitia, making a separation between them very difficult. The media was much thicker than normal due to the increased fibrous tissue. Normally, a mild fibrosis can be observed in the small arteries of nonhypertensive individuals during the third decade of life; however, it never appears as extensively as in the cerebral vessels of these cases of rapidly progressive hypertension. The medial elements of many of the arteries showed either a diffuse or a patchy hyalinization and loss of their tinctorial properties. These changes seemed to begin in the outer portion of the media and then to spread into entire segments of the vessel wall. Often the hyalinization was complete, replacing all elements (Fig. 1). This hyalinization is very unusual at such an early age since normally it does not occur in the cerebral arteries until the fifth or sixth decades.

Adventitia. The adventitial changes resembled closely those described in the media. In most vessels the adventitia was thicker than normal.

The extreme fibrosis and hyalinization of these small arteries appeared to weaken them to such an extent that erythrocytes often broke through the frayed elastica and fibrosed media and escaped into the perivascular space where they formed a ring-hemorrhage around the vessel.

The 7 remaining cases in this age group were from individuals suffering from chronic hypertension. There was no striking alteration in any of the small cerebral arteries. In an occasional artery there was seen a mild fraying of the elastica interna. Usually, however, this membrane appeared as a deeply staining, thick, compact, laminated structure with only a few regular waves and no signs of reduplication. The media of these vessels was also uninvolved. Although, as is normally the case, the bulk of this layer was composed of collagenous tissue, still the muscular elements were surprisingly prominent. In some of the arteries it appeared that even the normal degree of fibrosis was lacking and the vessels seemed more compact and muscular than is usually the case. Likewise, the adventitia was unchanged. Hyalinization and tinctorial alterations were minimal in this group of cases, although occasionally a mild patchy homogeneity appeared in a few of the collagenous fibers.

From these observations it can be concluded that in this early age group, patients with so-called malignant hypertension present most extensive alterations in the small cerebral arteries, while in the more slowly progressive and chronic cases the arteries are entirely free of visible alteration. In fact, in many vessels in those of the latter group the normal vascular fibrosis is retarded by the hypertensive process.

AGE GROUP 41 TO 60 YEARS

Thirty cases were available for study. In these the findings were much more difficult to evaluate, since normally in this age group extensive changes occur within the small cerebral arteries in the form of reduplication and fraying of the elastica, and fibrosis and hyalinization of the media and adventitia. In none of the hypertensive cases were the alterations in the vessel walls any different from those in normal individuals. Probably the most common observation was a fraying of the elastica interna. The media in most cases contained very little muscle tissue. It was composed almost entirely of collagen and showed a moderate degree of hyalinization. The adventitia was moderately thickened, often frayed and partially hyalinized. Many of these small arteries were surrounded by red cells that had passed through the weakened, frayed elements of the wall and had accumulated within the perivascular spaces.

In a few cases the small arteries were conspicuous by the absence of even those changes normally expected for the age. The elastica was not reduplicated and even its fraying was minimal or entirely absent. Although these vessels were composed predominantly of collagen, throughout this connective tissue there were varying amounts of obliquely arranged muscle cells (Fig. 2). The muscle elements were naturally quite irregular in occurrence but were definitely more prominent than in normal vessels of the same age group. The adventitia was unaltered. Tinctorial changes or hyalinization was not observed. The vessel walls were not thickened and their lumens were not narrowed.

AGE GROUP 61 TO 90 YEARS

Thirteen cases were studied in this group. In these the small arteries showed the same changes as are seen normally at this age.

Reduplication and fraying of the elastica were very pronounced. The media was composed almost entirely of loose bands of collagenous fibres. Curiously enough, in an occasional case even in the eighth decade, the arteries contained a few muscle fibers and cells scattered irregularly through the vessel wall. Their presence was never observed in the vessels of the control series. Tinctorial impairment was very frequent in this group. These tinctorial changes seemed to begin in the center of the vessel wall and gradually spread in all directions to involve large segments of the wall. In some cases only a faint outline of the original vessel could be made out. Hyalinization was also extensive and seemed to follow the same pattern as the tinctorial changes.

In view of the striking absence of definite alterations in the small arteries of these patients with long-standing hypertension, a study was made of the larger cerebral vessels in some of the same cases. Only those were studied in which the smaller vessels were exceptionally free of change. In direct contrast to the smaller arteries, the larger ones (over 300 μ in diameter) showed most extensive alterations, primarily in the form of severe intimal and medial changes. Areolar tissue eventually underwent degeneration with hyalinization. This process produced a marked irregular narrowing of the vessel lumen and in many of the vessels an extensive reduction of the blood flow through the vessel (Fig. 3).

DISCUSSION

In the present study, one is immediately impressed by the paucity of the actual alteration occurring within the small cerebral arteries in cases of long-standing hypertension. In fact, it appears that in certain cases the structure of such vessels in hypertensive patients is spared the routine wear and tear that occurs with increasing age. On the other hand, the larger arteries show definite and often far advanced arteriosclerotic changes with a marked narrowing of their lumens. It is generally believed that protracted hypertension might favor the development of a fairly severe arteriosclerosis, even though in many cases the blood pressure may be elevated for many years without resulting in excessively severe sclerotic changes. In an attempt to explain or correlate the paucity of changes in the smaller arteries and the exten-

sive changes in the larger ones, one could assume that, as the larger vessels become sclerotic and narrowed, there results a reduced blood pressure in the smaller arteries with a corresponding reduction in the wear and tear upon their walls. This might help preserve their normal architecture even in the face of a long-standing hypertension. A phenomenon of this type is not without its parallel in the human body. It has already been shown by Zon⁸ that in severe aortic stenosis the base of the aorta shows much less sclerosis or other change associated with age than is normally the case. These aortas have an elasticity corresponding to that of individuals 20 years younger. It was assumed by Zon that the narrowed aortic orifice protected the base of the aorta from the usual wear and tear to which it was exposed.

The present findings may also lend further evidence on the question whether the hypertension or the vascular pathology is the primary event. In view of the above findings it becomes apparent that, as far as the cerebral arteries are concerned, hypertension may exist for years with little or no effect upon the small arteries.

Attention may also be called to the fact that in some of our patients death was due to uremia; correspondingly, definite changes had occurred within the arterioles of the kidney and yet similar changes within the small arteries of the brain were not present. Apparently the arteriolar changes throughout the various organs of the body are not correlated.

CONCLUSIONS

1. The average small cerebral artery in cases of long-standing hypertension shows very little structural alteration.
2. The larger arteries often present definite arteriosclerotic changes with a marked narrowing of their lumens.
3. It is possible that the narrowing of the larger vessels reduces the wear and tear upon the smaller ones, thus preserving their normal architecture in the face of the long-standing hypertension.
4. In so-called malignant hypertension, the small arteries show extensive alterations. The elastica interna becomes reduplicated and the media undergoes a rapid fibrosis and patchy hyalinization.

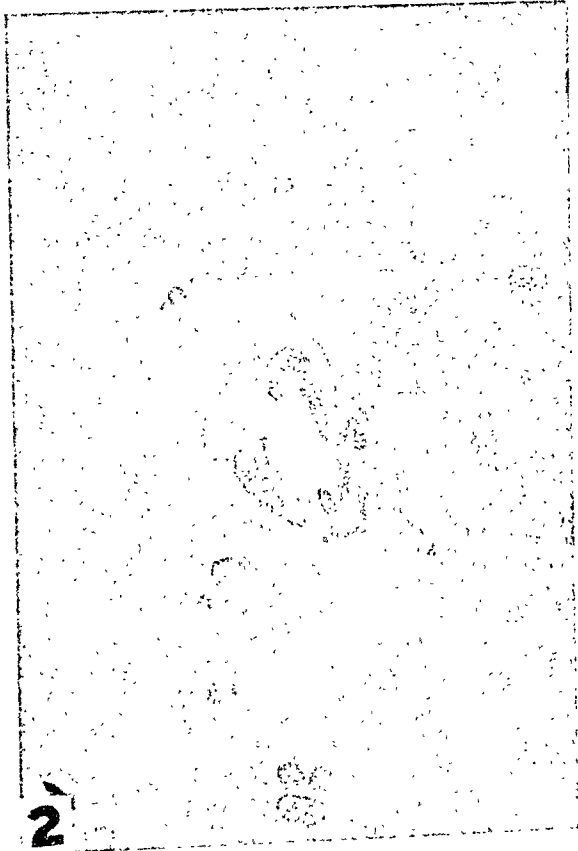
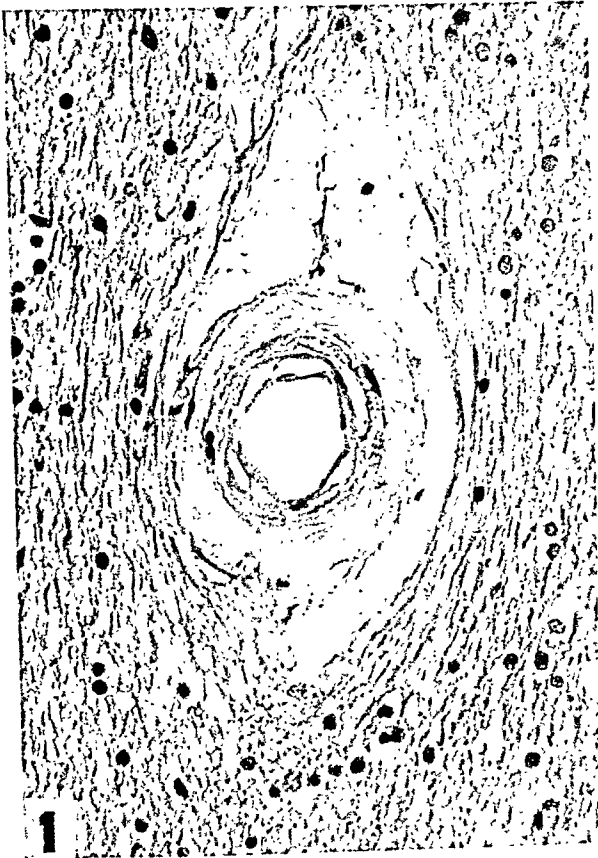
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DESCRIPTION OF PLATE

PLATE 9

- FIG. 1. Hyalinization and tinctorial alterations of a small cerebral artery in a case of malignant hypertension. The hyalinization is complete, replacing all of the wall elements. There is some reduplication and fraying of the intima. Hematoxylin and eosin stain. $\times 300$.
- FIG. 2. Structure of a small blood vessel from a chronic hypertensive patient in the fifth decade of life. A few muscle nuclei are still present within the wall elements. Hematoxylin and eosin stain. $\times 400$.
- FIG. 3. Structure of a large blood vessel from a chronic hypertensive patient. Note the severe degree of sclerosis. The vessel lumen is greatly reduced in size. Hematoxylin and eosin stain. $\times 200$.



Arteries in Hypertension

NECROSIS OF THE BONE MARROW WITH FAT EMBOLISM IN SICKLE CELL ANEMIA*

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INTRODUCTION

Fat embolism has been described in a wide variety of clinical conditions. The importance of this complication in instances of trauma to bone or fatty tissues is well recognized. Few have agreed, however, that the finding of fat emboli in other conditions is of any practical importance.

A number of investigators have shown that the examination of routine necropsy material for fat embolism will reveal positive findings in a certain proportion of the cases. Lehman and McNattin¹ found varying degrees of fat embolism in the lungs in 37 of 50 autopsies. The embolism was described as moderate to marked in 13 instances, 6 of which were unassociated with trauma. More recently, Vance,² in a study of 246 necropsies, found "very slight fat embolism" in only 7 of the 82 cases which were unassociated with trauma. His conclusions were in accordance with the much earlier observations of Warthin,³ who stated that in nontraumatic cases "the fat is so small in amount and the lesions so few, as to be of pathologic interest only."

We have recently had opportunity to study a case of sickle cell anemia in which the clinical picture and the pathological findings leave no doubt that fat embolism was an important factor in bringing about the patient's death. Groskloss⁴ and Warthin³ stated that fat embolism occurs in certain anemias. We have been unable, however, to find any report of a case similar to ours.

REPORT OF CASE

Clinical History. (N. Y. H. No. 59202.) The patient was a Greek housewife, 49 years old, who was admitted to the New York Hospital on four occasions.

Family History. The available family history was meager, but both of the patient's parents were said to be dead of causes unknown. They were born

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in Greece, of Greek parentage. There was no known history of familial disease.

Past History. The patient's general health was said to have been good, except for "nervousness," until March 1934, when she was admitted to the Surgical Service. She had been awakened from her sleep by a severe pain said to have been present throughout her entire body, but most severe in the right upper quadrant of the abdomen. The onset of the pain was associated with vomiting; attempts to drink water or to take food were followed by further vomiting. The pain was constant in nature and unrelieved by heat. The patient's husband stated that she had had a similar attack 3 years before, but none before or after to his knowledge. The only positive physical findings were: anemia, slight icterus, slight spasm and definite tenderness in the right upper quadrant.

A tentative diagnosis of acute cholecystitis was made and a laparotomy was performed. No stones were present and the gallbladder was not inflamed. No lesion was found to explain the patient's symptoms. The patient received four transfusions with a total of 1450 cc. of blood. She was discharged on April 15, 1934.

The patient was brought to the Emergency Pavilion on June 9, 1934, complaining of pains "like pins and needles" over the entire body. The patient was highly excitable but the physical examination was otherwise negative. She was given phenobarbital and sent home.

The patient was admitted to the Gynecological Service on January 22, 1935 because of metrorrhagia. Dilatation and curettage were done. The endometrial tissue removed was that of a postmenopausal uterus.

On October 5, 1935 the patient was again seen in the Emergency Pavilion. She complained of severe pain "just like last time," associated with nausea and vomiting. The patient was moaning, screaming, and lashing about in bed. She was admitted to the Surgical Service where she was observed for 24 hours. The pain subsided and in view of an entirely negative physical examination the patient was discharged.

The patient got along well until 1939. In that year she had several episodes of pain, nausea and vomiting, associated with "darkening of the skin."

Present Illness. At 2 a.m. on January 1, 1940 the patient was awakened from her sleep and caused to cry out by excruciating pain in the lumbar region of the spine. The pain returned in paroxysms and became generalized. The paroxysms of pain caused the patient to "break out in cold sweat." The pain was associated with vomiting and the patient continued to vomit all ingested food or water up to the time of admission to the Medical Service on January 4. On the day before admission the patient had severe shaking chills; her temperature rose to 102° F. and she became comatose.

Physical Examination. Temperature 39° C. Pulse 122. Respirations 38. Blood pressure 132/64. The patient was obviously critically ill. She was semicomatose. The neck was slightly stiff. There were petechiae in the conjunctivae and skin. The spleen was palpable. The liver was slightly enlarged. There was questionable icterus.

Laboratory Findings. These are summarized in Table I.

Course and Treatment. Throughout the patient's hospital stay her temperature varied from 38.6° to 40° C. The patient was at all times semistuporous. She was given six blood transfusions, totalling 2250 cc. of

TABLE I
Laboratory Findings in Case Reported

	Admission												
	I (1934)					II (1935)	III (1935)	IV (1940)					
	3/19	3/23	3/24	3/26	3/29	1/22	10/5	1/4	1/6	1/8	1/9	1/12	1/13
Date													
R. b. c. (millions per cu. mm.)	2.7	1.9	2.4	3.2	3.6			2.3	3.1	3.7	3.4	3.5	3.6
Hb. (14.5 gm. per 100 cc. = 100%)	55%	38%	48%	60%	70%	74% 33%	85%	41% 15%	50%	60%	60%	60%	66%
Cell volume													
Sickle cells			+	+	—			+	+	+	+	+	+
Reticulocytes			+	+				+	+	+	+	+	+
Anisocytosis			+	+				+	+	+	+	+	+
Poikilocytosis			+	+				+	+	+	+	+	+
Nucleated r. b. c. (% of w. b. c.)													
W. b. c. (corrected)	8950	23,300	23,000	67%		3250	7200	6%	12,000	35%	31%	22%	15%
Adult polys	26%			20,000				11,700	8500	8500	7100	6500	7600
Immature polys	52%			23%				50%	60%	57%	55%	34%	21%
Lymphocytes	15%			50%				15%	18%	9%	11%	43%	47%
Platelets				11%				29%	20%	23%	27%	22%	30%
Bleeding time*				260,000									
Clotting time**				3 min.									
Fragility				4 min.									
Sedimentation rate†					Normal				3.5 min.				
Icteric index	19	36	25	23	19	0.1		0.05	9 min.		0.1		

* Duke method: Normal = 1 to 3 minutes

** Lee and White: Normal = 5 to 10 minutes

† Rourke and Ernste: Normal = 0.08 to 0.35 mm. per minute

citrated blood. She was unable to take food by mouth and was given repeated infusions of 5 per cent glucose in saline solution. The petechiae noted on admission gradually faded and no new ones appeared. Her neck remained stiff but no Kernig or Babinski signs were elicited. The spinal fluid pressure was 140 mm. of water, the fluid was clear and there were three lymphocytes per cu. mm. Cultures of the fluid were sterile. The protein was 40 mg. per cent; sugar 92 mg. per cent; chlorides 740 mg. per cent; and the Wassermann negative. The patient's condition remained unchanged until the tenth hospital day, when she became more deeply comatose and her respirations became rapid and shallow. The blood pressure fell to 90/60. She became deeply cyanotic. The patient was placed in an oxygen tent and given respiratory stimulants but these were of no avail and the patient expired a few hours later.

POSTMORTEM EXAMINATION

The description will be confined to the positive findings.

Macroscopic Examination. The *spleen* weighed 350 gm. It was adherent to the adjoining structures but was easily separated. The capsule was pale green in color and slightly wrinkled. On the surface were many gray areas with irregular "map-like" boundaries. These varied from a few millimeters to 2 cm. in diameter. The spleen was moderately firm but definitely "lumpy" in consistency. The "lumpy" areas of increased density corresponded to the gray areas described above. The spleen cut with a gritting sensation. The cut surface was dark red in color except for gray areas similar to those described on the surface. These occupied approximately 25 per cent of the cut surface. The *liver* weighed 1610 gm. Many pale, roughly circular areas, less than 1 mm. in diameter, were apparent on the cut surface of the liver. The *kidneys* each weighed 160 gm. The glomeruli stood out prominently as tiny hemorrhagic spots on the capsular and cut surfaces. The *bone marrow* was pale in color. The *arachnoid* of the interpeduncular space was slightly thickened. There was widespread cortical atrophy of the *brain*.

Microscopic Examination. The capsule and trabeculae of the *spleen* were moderately thickened by collagen fibers, between which were clusters of golden brown refractile bodies, roughly cylindrical in outline and in many instances segmented so as to resemble "bamboo poles." These bodies were made up chiefly of iron pigment. Similar masses of iron pigment and collagen fibers were present throughout the pulp. Such nodules were surrounded by dilated sinusoids containing many sickle cells and macro-

phages. The macrophages were loaded with erythrocytes and iron pigment. All of the malpighian corpuscles appeared to be involved. There were pale-staining areas of necrosis scattered irregularly through the pulp. The blood vessels were surrounded by collagen fibers and iron pigment but no thrombi were found.

The sinusoids of the *liver* were congested. The adjacent liver cells were extensively vacuolated and contained an unusually large amount of bile pigment. There were many bile thrombi in the biliary canaliculi. Small focal areas of necrosis of liver cells were present.

Material obtained by aspiration biopsy of the *sternal marrow* before death was necrotic and could not be stained satisfactorily. Sections prepared from postmortem material were also characterized by widespread necrosis with marked reduction in the blood-forming constituents and fat. The necrotic areas consisted of a faintly eosinophilic network in which were scattered granular debris, occasional polymorphonuclear leukocytes and numerous macrophages loaded with fat and chromatin particles. Foci of erythrocytogenesis contained mature cells, many of which were sickle shaped. Myelogenesis was normal. The megakaryocytes were reduced in number.

In the *brain* were many small areas of focal necrosis, both in the gray matter and in the white matter (Fig. 1). In the middle of some of these foci a few red blood cells were present. In addition there were many small hemorrhages, both in the cerebral cortex and in the cerebellum. These hemorrhages were usually perivascular. In these areas many of the red blood cells showed extreme degrees of sickling (Fig. 2). Wherever hemorrhage had occurred there was considerable proliferation of microglia cells although none had yet attained the form of gitter cells. Associated with this was a definite increase in the number of astrocytes in these areas. There were many fat emboli throughout the brain and in many instances the small areas of focal necrosis showed a capillary, either in the center of the area or just to one side, filled with droplets of fat (Fig. 3).

Frozen sections of lung, liver, kidney and spleen stained for fat contained many droplets in the arterioles and capillaries (Figs. 4 and 5).

Anatomical Diagnosis. Sick cell anemia; splenomegaly (350

gm.) with areas of necrosis and siderofibrotic nodules; icterus; bile pigment thrombi in biliary canaliculi; focal necrosis of the bone marrow; fat emboli in lungs, brain, liver, spleen and kidneys; disseminated focal necrosis of the brain due to fat emboli.

DISCUSSION

Sickle cell anemia is rare in the white race and it is quite unusual for patients more than 30 years of age to present clinical evidences of this disease. The diagnosis of sickle cell anemia in this instance, however, is based upon clear-cut clinical and anatomical findings. The history of repeated bouts of pain, both abdominal and muscular, with vomiting and "darkening of the skin," is typical. These were associated with a normocytic anemia, leukocytosis, mild icterus, large numbers of nucleated red blood cells, and a normal bleeding and clotting time. The sickling phenomenon was so prominent as to be manifest even in routine blood smears on several occasions. The anatomical changes in the spleen were precisely those described by Diggs.⁵ The finding of large numbers of sickle cells in the sections further corroborates the diagnosis.

There are certain respects, however, in which this case differs from previously described cases of sickle cell anemia. The clinical history suggests that the terminal attack began in the same manner as the previous ones. On the third day, however, the patient developed chills and fever, became comatose, and presented numerous petechiae over the skin and in the conjunctivae. The petechiae faded and no new ones appeared but the patient remained comatose. It was on the third day, no doubt, that the blood was "showered" with fat emboli. The signs and symptoms a few hours before death suggest a second "shower" of emboli. The clinical picture is explained thereby and the post-mortem findings are compatible with this interpretation.

The source of these fat emboli is not entirely clear. The bone marrow was necrotic, as has been previously described in cases of sickle cell anemia,^{6,7} and it seems probable that this was the source of the emboli. In recorded instances of fat embolism, necrotic purulent foci and septic processes of the bone marrow have been described as the source of fat emboli by some of the early observers.⁸ Lehman and Moore,⁹ on the basis of *in vitro*

experiments, concluded that fat embolism might be produced readily in the bone marrow on a nontraumatic basis purely by the absorption of histamine from injured tissue into the blood stream.

The central nervous system involvement in this case is of particular interest. Several observers have previously described central nervous system lesions. Indeed, Bridgers¹⁰ has pointed out that signs and symptoms of cerebral vascular thrombosis or intracranial hemorrhage may be the first manifestations of sickle cell anemia. Sensory and motor disturbances, headaches, nausea and vomiting, and signs of meningeal irritation are frequently reported.¹¹ The lesions of the nervous system have been inadequately described in most instances. Bridgers¹⁰ described obliterative vascular lesions in one case. In another he reported the finding of multiple focal areas of necrosis and hemorrhage, apparently similar to those described in our case. The clinical picture in the two cases is also very similar.

The possible rôle of trauma or some toxic agent has been studied carefully. No evidences that either factor was involved could be obtained from careful questioning of the patient's family, or from the necropsy findings. Inasmuch as the clinical picture was fully developed prior to the patient's admission to the hospital, and in view of the glial proliferation found in the central nervous lesions, it is improbable that any manipulations such as venepunctures or subcutaneous injections following admission to the hospital played any important rôle in producing the lesions described. The similarity of the complaints and of the laboratory findings on each of the several admissions makes it improbable that toxic damage to the bone marrow by some extraneous substance need be considered.

SUMMARY

A case of sickle cell anemia in a Greek housewife, 49 years old, is described. The known clinical history of acute exacerbations is of 6 years' duration. The terminal episode is characterized chiefly by cerebral manifestations which are adequately explained by the presence of widespread focal areas of hemorrhage and necrosis in the nervous system. These result from fat emboli which we believe to be secondary to necrosis of the bone marrow.

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DESCRIPTION OF PLATES

PLATE 10

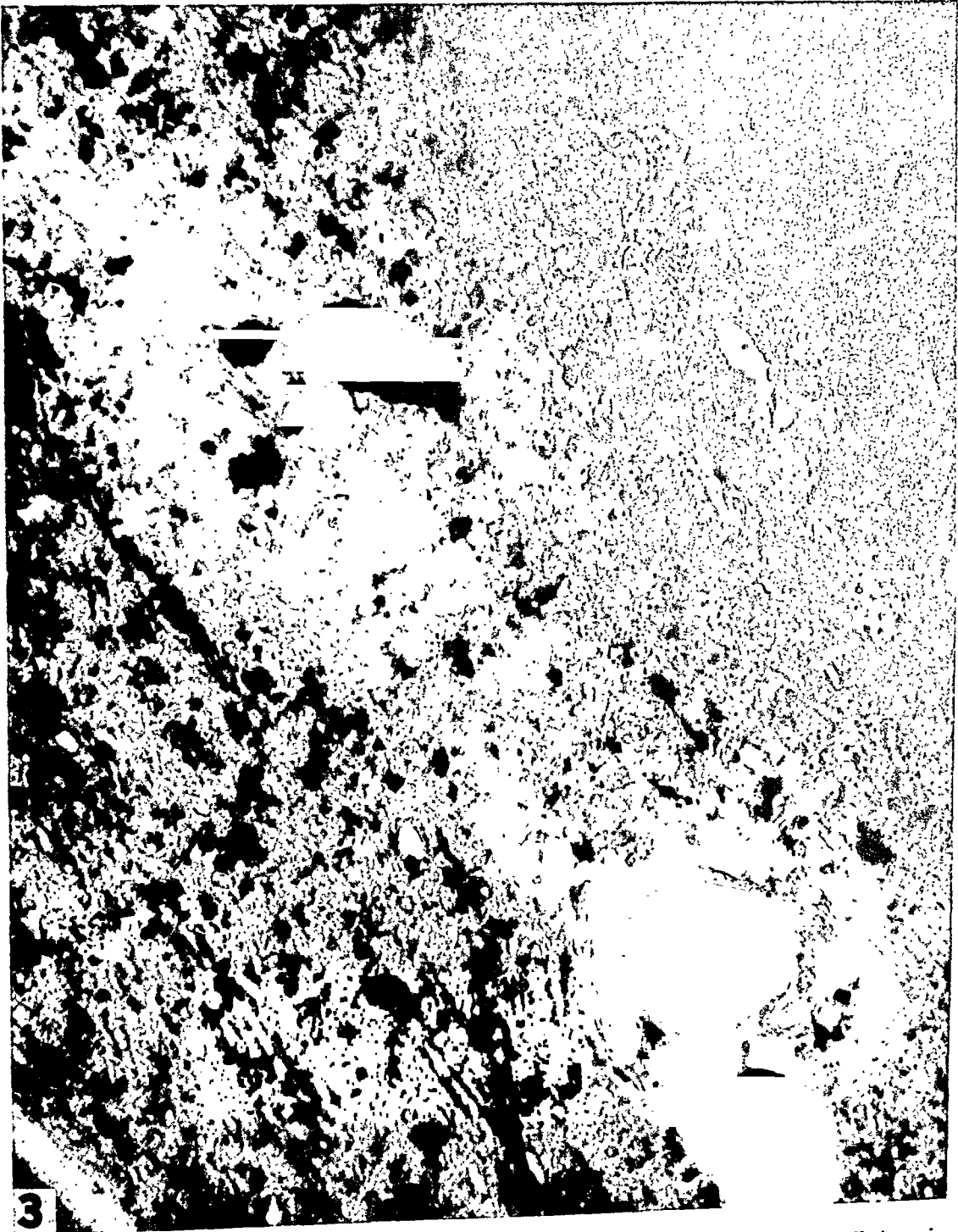
FIG. 1. Section of cerebral cortex (Loyez' stain for myelin sheaths) showing focal necrosis of the white matter. $\times 190$.

FIG. 2. Hemorrhage near wall of the third ventricle (Loyez' stain for myelin sheaths) showing sickle cells. $\times 1500$.



PLATE II

FIG. 3. Section of superior frontal gyrus of brain (Marchi's stain) showing fat emboli in capillaries surrounded by area of necrosis. $\times 360$.



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Fat Embolism in Sickle Cell Anemia

Wade and Stevenson

PLATE 12

FIG. 4. Section of lung (Sudan III stain) showing fat emboli in the capillaries. $\times 650$.

FIG. 5. Section of kidney (osmic acid stain) showing fat emboli in the glomerular capillary loops. $\times 650$.



Fat Embolism in Sickle Cell Anemia

ISOLATION OF THE VIRUS OF HERPES SIMPLEX
AND THE DEMONSTRATION OF INTRANUCLEAR INCLUSIONS
IN A CASE OF ACUTE ENCEPHALITIS*

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Although there is an extensive literature pertaining to the virus of herpes simplex as an etiological agent of encephalitis in man, there is no reported case of fatal encephalitis from which the herpes virus has been isolated which has shown intranuclear inclusions in the brain resembling those of herpetic lesions. In the first report of the Mathewson Commission,¹ a summary of the literature concerning the relation of the herpes virus to encephalitis mentioned only 9 instances of the isolation of a virus identified as that of herpes simplex from cases of encephalitis. In 5 of these, the virus was isolated from the brain; in 3, from the spinal fluid; and in 1, from the nasopharynx.

Subsequent to this report, Gay and Holden² isolated a virus from the brain of a man who died during an acute exacerbation of chronic encephalitis. They considered it identical with the herpes virus. A second virus, isolated by Gay and Holden² from the brain of a laboratory worker, 27 years old, who died following the bite of a monkey, was likewise reported as the virus of herpes simplex. However, it seems that the latter virus, also isolated and described by Sabin,³ was not the virus of herpes simplex but that now designated as virus B. Also, with brain material from 3 cases of encephalitis, all occurring in children following measles, Gay and Holden² produced in rabbits, skin lesions which were in keeping with those of herpes. In none of these 3 cases was the virus identified as that of herpes.

Other investigators⁴⁻⁸ have reported the presence of an agent which they considered the virus of herpes, although not definitely established as such, in the brain or in the spinal fluid of patients

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with symptoms of encephalitis or meningitis. The methods used in these investigations to demonstrate the presence of herpes virus were the production of an encephalitis in rabbits following the intra-ocular or intracerebral inoculation, or the production of corneal lesions alone following intra-ocular inoculation. The accuracy of the latter method has been criticized by Rupilius and Szekely,⁹ who questioned the criteria used for judging a positive corneal reaction for herpes virus. They were able to produce a punctate keratitis, similar to that described by some investigators as a positive reaction for the herpes virus, by inoculation of non-specific solutions.

In addition, the etiological significance of the herpes virus, when found in the brain or spinal fluid, has been questioned because of its occasional demonstration in the spinal fluid of individuals showing no evidence of encephalitis or herpetic eruptions. Such instances, however, are less frequent than is commonly supposed, and according to Doerr and Hallauer¹⁰ latent infections of the central nervous system with herpes virus never have been proven. In 1937 Zurukzoglu¹¹ summarized the reports of results obtained by a number of investigators with spinal fluid from individuals showing no evidence of encephalitis or herpetic eruptions. In five different investigations, in which a total of 108 spinal fluids were tested for the presence of herpes virus, the results were entirely negative. Zurukzoglu cited the following investigations in which positive results were obtained. The herpes virus was isolated from only 1 of 100 spinal fluids examined by Flexner and Amos. Of 50 fluids examined by Zurukzoglu, 2 gave positive results. Zurukzoglu also tested the spinal fluid from 8 cases, without clinical symptoms of encephalitis, shortly after the occurrence of herpes labialis. Two of these gave evidence, by intra-ocular inoculation of rabbits, of herpes virus in the spinal fluid. The only results reviewed, which differed strikingly from the others, were those of Bastai and Busacca who reported the presence of the herpes virus, demonstrated by intra-ocular inoculation of rabbits, in 18 of 22 spinal fluids from unselected cases.

In addition to these reports of the demonstration of a virus in the brain or spinal fluid, there are a number of reports^{12,13} of the demonstration of a virus which was capable of producing keratitis and/or encephalitis by corneal inoculation of rabbits in

the nasopharynx or saliva of patients with clinical symptoms of acute or chronic encephalitis. The etiological significance for encephalitis of the herpes virus thus obtained may be questioned in view of the frequent occurrence of herpes infections and also in view of the demonstration of the herpes virus in the saliva of normal individuals.¹⁴

In 1933 Dawson¹⁵ reported a case of fatal lethargic encephalitis in a boy 16 years of age. Sections of the brain showed, in addition to minute hemorrhages and perivascular infiltration of lymphocytes, intranuclear inclusion bodies in the cerebral lesions comparable to those seen in herpetic infections. In this instance no etiological agent was isolated. To our knowledge this is the only description in the literature of intracellular changes in the brain lesions of human encephalitis suggestive of those produced by the herpes virus. However, these lesions were believed not to be identical with those of herpes simplex because the nuclear inclusions were never of the very large granular type seen in herpes.

It is the purpose of this paper to present a case of encephalitis occurring in an infant 4 weeks old in which intranuclear inclusion bodies, compatible with those of herpes, were found in the cerebral lesions and from which a virus, identical with that of herpes simplex, was isolated from brain tissue. Because of the importance of establishing the identity of the etiological agent, a detailed account of its investigation is given.

REPORT OF CASE

Clinical History and Observations. The patient, a white male infant 4 weeks old, was admitted to St. Louis Children's Hospital because of irritability, refusal to nurse, and twitching of the left side of the body.

No family history of significance was obtained. The infant was born at home 1 month prematurely; the delivery was normal. Until 4 days before entry to the hospital he had been breast fed, had developed normally and appeared in good health. At that time, however, it was noticed that he was irritable and fretful, but there was no noticeable fever and no vomiting. Two days before admission to the hospital he began to complain when moved and to scream out suddenly. On the day before admission, twitching of the left arm and leg was first noticed and the child cried continuously. On the day of admission the twitching of the left side of the body continued, the child refused to nurse and had a slight fever.

The patient appeared moderately well nourished; he was listless and was having occasional twitches of the left arm and leg. The fontanelle was full

and tense. Both ear drums were dull, and the left one bulging. To external examination the eyes were normal except for a slight clouding of the cornea. Examination of the eye grounds disclosed a pale zone about the optic disks; however, a diagnosis of optic atrophy was considered questionable. The tongue and mucous membranes of the mouth appeared normal. The pharynx was not hyperemic. A seborrheic dermatitis was present on the scalp, but no other abnormalities of the skin were observed. The white blood cell count was 18,000; the red blood cell count, 4,100,000; and the hemoglobin content of the blood, 11 gm. per 100 cc. The temperature on admission was only slightly elevated and later dropped to subnormal levels.

As a result of the first lumbar puncture, bloody, sterile fluid was obtained. Several other attempts were made to obtain spinal fluid from the cisterna and from the ventricles, and a small amount of blood-tinged cisternal fluid was obtained on one occasion.

The child's illness steadily progressed. The fontanelle became more and more tense; the convulsive movements of the extremities continued; and there was a generalized convulsion on the day after admission to the hospital. Death occurred on the fifth hospital day. A clinical diagnosis of acute encephalitis was made.

POSTMORTEM EXAMINATION

A complete autopsy revealed no macroscopic abnormalities other than those in the brain. On gross examination of the brain the leptomeninges appeared hyperemic; and in the region where the intraventricular punctures had been made there were a few small clots of blood, but no extensive hemorrhage. Material was taken from the frontal cortex for animal inoculation. Nothing unusual was observed when a cut was made through this portion of the brain; however, the entire brain was unusually soft, even for that of a young infant. Unfortunately, only the cerebellum and the brain stem were saved for microscopic study.

The microscopic study of organs other than the brain revealed nothing abnormal.

Sections from the pons, medulla, and cerebellum were stained with hematoxylin and eosin, phloxine-methylene blue, and with bacterial stains. There was an extensive inflammatory process involving the brain tissue and, to some extent, the meninges. In certain areas the meninges were entirely normal, while in others, always in association with changes in the underlying brain tissue, there was an infiltration of cells concentrated about blood vessels. These cells were chiefly lymphocytes and larger mononuclear cells. Only an occasional polymorphonuclear leukocyte was seen. The walls of the meningeal blood vessels were not damaged.

In general, the changes seen in the sections from the three parts of the brain were alike, but less severe in the sections from the cerebellum. Perivascular infiltration of cells like that occurring in the meninges was conspicuous within the brain substance. The vessel walls, however, appeared normal except that the lining endothelial cells were often unusually large. There were focal accumulations of cells having either round or elongated nuclei: some with indistinctly outlined cytoplasm, others with sharply outlined cell margins (Fig. 1). These focal accumulations of cells closely resembled the focal lesions, largely of microglial origin, seen in other types of virus encephalitis. In some instances they were associated with degenerating nerve cells. In other microscopic fields a more diffuse inflammatory reaction of mononuclear cells occurred, involving, in some instances, groups of large ganglion cells which showed stages of degeneration. Isolated degenerating nerve cells surrounded by a cluster of small mononuclear cells were also seen. In sections from both the pons and medulla, areas of necrosis were present which were slightly larger than a low power field (16 mm. objective). This necrosis was not localized about vessels. In fact, the vessels were remarkably well preserved, even in the necrotic zones. One necrotic area had undergone liquefaction (Fig. 2); in the others there were many large fat-holding phagocytes and remnants of necrotic brain tissue.

In the cerebellum the Purkinje cells appeared normal except in localized areas where they, together with the adjacent small nerve cells, were undergoing necrosis.

Intranuclear Inclusions. The degenerative changes in individual nerve cells were especially interesting. Some of these cells showed pyknotic nuclei and deeply stained, shrunken cell bodies; others were poorly outlined and showed varying degrees of karyolysis. Many cells, however, showed more specific nuclear changes in the form of intranuclear inclusions which varied somewhat in appearance. In some nuclei the chromatin was margined at the nuclear membrane about an acidophilic central body (Figs. 3 and 4). The smaller of these central bodies were four to five times the usual size of a nucleolus. Occasionally, there was a fine network of light blue-staining material which radiated from the acidophilic central body to the basophilic margin. In other

cells a clear area surrounded the acidophilic inclusion and at times a small, basophilic nucleolus was seen in addition to the margined chromatin. Many of the inclusions conformed in shape to the general contour of the nucleus which contained them. Other nuclei had a different appearance. In these there was a very deeply stained margin of chromatin arranged in dots, while the rest of the nucleus was completely occupied by a material which stained lilac with phloxine-methylene blue and was often definitely granular (Figs. 5 and 6). Nuclear changes of both types were interpreted as intranuclear inclusion bodies corresponding to forms seen in known herpetic lesions.

ISOLATION OF THE VIRUS

A piece of cortex was ground without abrasive in a small amount of nutrient broth. After light centrifugation, 0.03 cc. of the supernatant fluid was inoculated intracerebrally into 6 Swiss mice. On the third day after inoculation, 4 of the 6 mice were found dead and the remaining 2 were observed in convulsions. The brains of these mice were removed aseptically, cultured, and passed to other Swiss mice, each of which received 0.03 cc. of a 10 per cent dilution of the brain material; these animals died or were killed, after being observed in convulsions, on the third day following inoculation. The infective agent has been maintained in Swiss mice until the present, and the brains of mice have been used as the source of material for studying its characteristics.

To determine the nature of the infectious agent, aerobic cultures of the human brain were made in dextrose infusion broth and both aerobic and anaerobic cultures of the infected mouse brains were made in broth and on blood agar. No organisms were grown. Sections from the human brain and from the brains of experimental mice, stained by the MacCallum-Goodpasture method, were studied for bacteria. None could be demonstrated. The infectious agent was therefore considered to be a virus and, as a matter of convenience, was designated "R. T." virus.

Infected mouse brains from early passages were stored in 50 per cent glycerin in Locke's solution at 5° C. After 5½ months the virus from the second mouse brain passage remained active, apparently retaining its original infectivity; a 10 per cent emul-

sion inoculated intracerebrally killed mice in slightly less than 3 days.

The filtrability of the virus was tested as follows. A 10 per cent suspension of infected mouse brain was made in 2 per cent normal horse serum broth, centrifuged at 2000 r.p.m. for 5 minutes in a horizontal centrifuge, and the supernatant fluid further diluted to 1 per cent with horse serum broth. This suspension was put through two new Berkefeld N candles, prepared, after washing, by filtering 30 cc. of 2 per cent normal horse serum broth through each. Neither filtrate was infectious for mice by intracerebral inoculation, while the unfiltered 1 per cent brain suspension was still infectious when diluted tenfold, all of 4 Swiss mice dying following intracerebral inoculation.

On a number of occasions, when the same procedure was carried out using nutrient broth as a diluent and for preparing Berkefeld N and V filters, no virus was demonstrated in the filtrates. These results are not surprising in view of the known difficulty in the filtration of herpes virus.

RESPONSE OF LABORATORY ANIMALS TO THE VIRUS

Mice. As already stated, mice succumbed in 3 days to intracerebral inoculation of 10 per cent emulsions of infected mouse brain; and the virus, maintained by mouse passage, was uniformly infectious by this route in dilutions as great as 10^{-4} . Following subcutaneous inoculation of as much as 0.25 cc. of a 10 per cent emulsion of infected mouse brain, an occasional mouse developed paralysis, but the great majority remained well.

After intracerebral inoculations of the virus the mice frequently showed little or no evidence of illness until they began to have active muscular twitchings or generalized convulsions. Death in severe convulsions usually followed shortly thereafter.

Lesions were not found in organs other than the central nervous system. In sections of the brain the most conspicuous lesion was a meningeal exudate extending into perivascular spaces within the brain. In some areas the exudate was composed of well preserved, small lymphocytes and slightly larger mononuclear cells. Other fields showed a partially necrotic meningeal exudate, in the debris of which lymphocytes and mononuclear cells, together with polymorphonuclear leukocytes, were still recogniz-

able. In the mouse brain, inclusions in the nerve cells have been but rarely observed.

Guinea Pigs. Of 8 guinea pigs inoculated intracerebrally with 0.15 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 6 remained well and 2 died. One of the latter, which died on the eighth day following inoculation, was autopsied. Microscopic sections of the brain showed a slight cellular infiltration in the meninges and perivascular spaces similar to that seen in the less severe reactions in the mouse brains. No intracellular inclusions were observed in several sections.

The corneae of 2 guinea pigs were scarified and rubbed with a 10 per cent emulsion of infected mouse brain. In each animal, beginning on the fourth day, a slight opacity of the cornea appeared. This opacity decreased after the seventh day, and neither animal showed other symptoms.

Three guinea pigs, inoculated intradermally with 0.2 cc. of a 10 per cent emulsion of infected mouse brain, developed, on the fifth day thereafter, small inflammatory nodules at the site of inoculation. After the ninth day these nodules regressed and the animals remained well.

Rabbits. Three rabbits, when inoculated intradermally, developed no lesions. Four rabbits were inoculated intracerebrally with 0.3 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain. Of these 4 animals, 2 remained well. A third showed convulsions on the twelfth day, and at that time an opacity of the cornea had also developed. The fourth was found dead on the fifth day.

Five rabbits were inoculated on the scarified cornea with approximately 0.1 cc. of a 10 per cent emulsion of infected mouse brain. Only 1 of these animals remained normal. Each of the other 4 developed an opacity of the cornea which appeared 6 to 11 days after inoculation and gradually spread to involve the entire cornea. In one instance, extensive ulceration of both the cornea and the conjunctiva occurred. One of the rabbits was killed and the eye removed for study as soon as the opacity appeared. In the other 3 animals which developed a keratitis, partial paralysis of the posterior extremities occurred 2 to 3 days following the first appearance of the keratitis. In 2 of these animals this paralysis was unilateral, on the same side as the infected

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that have been completely destroyed often contain fat in fine droplets.

The accumulation of fat in the cells of the glomeruli, as in those of the tubules, is due to injury which renders the affected cells incapable of utilizing fat in the normal manner. The injury may result either from the action of a toxin or some other poison, or from anoxia due to the marked narrowing of the afferent arteries.

In nephrosclerosis the fatty changes in the glomeruli appear to result entirely from ischemia, for in this condition we have not observed fat in glomeruli that were well supplied with blood even though the wall of the afferent artery was hyaline and contained much fat. The presence of fat in the glomeruli in this disease is accompanied by other changes in the capillary loops, ranging from simple collapse to partial or complete hyalinization or even necrosis. Since the blood is the only source of fat, it can accumulate only in glomeruli through which some blood, even if in greatly reduced amount, is still circulating. Under such a condition of anoxia the cells of the glomeruli may acquire fat, perhaps for the same reason that the muscle fibers of the heart become loaded with fat in severe anemias. Why the cells in the periphery of a glomerulus are the first to acquire fat, and the remainder become fatty only in extreme grades of the condition, is not known.

Fatty changes in the glomeruli are much less marked in glomerulonephritis than in nephrosclerosis. In glomerulonephritis the wall of the afferent artery is either entirely free from fat or contains only very minute amounts. Within the glomerulus the fat is most concentrated near the hilus and may be entirely limited to this region. When present in larger amounts it tends to follow the capillary loops radially toward the periphery of the glomerulus. It is generally believed that in this condition the glomeruli are damaged by toxic substances brought to them in the blood stream. A highly dilute colloidal poison in the blood plasma would become more and more concentrated as the blood traverses the capillary loops and would reach its greatest concentration at the efferent ends of these loops. The greatest injury to the cells would occur, and presumably the fatty degeneration would be most marked, at this place. This may account for the concen-

DESCRIPTION OF PLATE

PLATE 118

- FIG. 1. Moderate amyloidosis in the kidney (biopsy) of rabbit No. 9, injected with a scarlatinal streptococcus for 10 months and showing albuminuria for 3 months. The glomeruli contained amyloid in amounts varying from 1 to 4 plus. The proximal convoluted tubules are atrophic and the distal tubules markedly dilated. There is some increase in interstitial tissue. Hematoxylin and eosin stain. $\times 170$.
- FIG. 2. Marked amyloidosis in glomeruli, and degeneration of tubules in rabbit No. 3, injected with a "nephritic" hemolytic streptococcus for $3\frac{1}{2}$ months in two courses, with a total experimental period of $6\frac{1}{2}$ months and persistent albuminuria in the last $1\frac{1}{2}$ months. Congo red stain. $\times 170$.
- FIG. 3. Marked glomerular amyloidosis, tubular atrophy and obstruction, and interstitial fibrosis in rabbit No. 11, injected with a type I pneumococcus for 6 months. Albuminuria appeared at $2\frac{1}{2}$ months and persisted during the 5 months after the injection period. Hematoxylin and eosin stain. $\times 63$.
- FIG. 4. Marked glomerular amyloidosis and fibrosis of parenchyma in rabbit No. 312; injected with *Streptococcus viridans* for 4 months and surviving another 14 months, with albuminuria during most of this period. The spleen and liver showed only traces of amyloid. Van Gieson's stain. $\times 75$.
- FIG. 5. Medial necrosis and calcification, and intimal fibrosis in aorta of rabbit No. 535, injected with a "nephritic" hemolytic streptococcus for 7 weeks in two courses, with a total experimental period of 10 months. There was generalized amyloidosis. The aorta was calcified as far as the origin of the renal arteries. Hematoxylin and eosin stain. $\times 18$.
- FIG. 6. Atheromatous plaque in ascending aorta of rabbit No. 360, injected with a Friedländer bacillus during $1\frac{1}{2}$ months and surviving another 14 months. There was marked generalized amyloidosis, with fibrotic kidneys. The plasma cholesterol ranged between 179 and 352 mg. per cent for a year. Van Gieson's stain. $\times 250$.

tration of fat in the region of the hilus in acute infections, in glomerulonephritis and in the kidneys of dogs poisoned with minute doses of diphtheria toxin. The latter is believed to be a colloid, albeit of relatively small molecular size, but to which the glomerular filter is either not at all, or only very slightly, permeable. Fat in the glomeruli in diphtheria, scarlet fever, pneumonia, peritonitis and other acute infections is of the fine-droplet, hilar type.

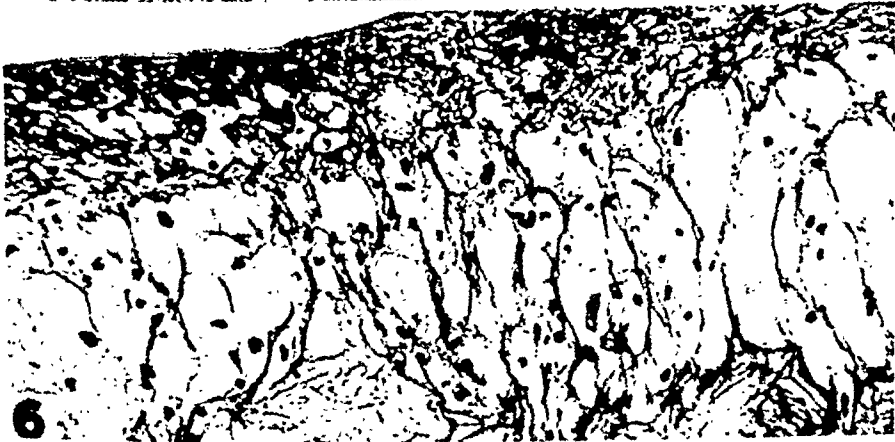
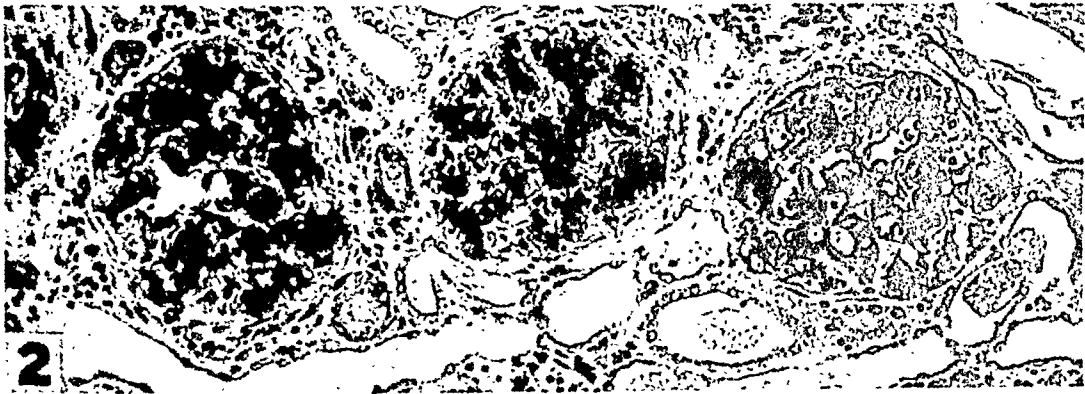
Proof that the fat in capillary walls near the hilus of a glomerulus is in the efferent ends of the capillaries is difficult to establish. It is not present in every glomerulus in a section. It is relatively easy to identify the afferent artery as it enters the glomerulus. Identification of the efferent vessel is far more difficult. In spite of prolonged search we have not been able thus far to find the desired combination of a glomerulus which contains fat in its hilar region and also shows the efferent vessel leaving its hilus. However, in many instances the afferent artery was traced into the glomerulus and the fat was found to be in the walls of capillaries on one or both sides of this vessel and apparently not directly associated with it. This suggests, although it cannot be said to prove, that the fatty changes were in the efferent ends of the capillary loops.

CONCLUSIONS

1. Fatty changes occur in the glomeruli in acute infections such as diphtheria, pneumonia and peritonitis; in acute and chronic glomerulonephritis; and in nephrosclerosis.

2. In acute infections and in glomerulonephritis the fat is most abundant in the immediate vicinity of the glomerular hilus and may be limited to this region. When present in greater abundance it tends to extend radially along the capillary loops, having much the same distribution as the hyalinization and thickening of the basement membrane in chronic glomerulonephritis. In the early stages it consists of very fine dustlike particles; with increase in amount the droplets also increase in size. The afferent artery is free from fat.

3. In nephrosclerosis the walls of the afferent arteries contain much fat which stops at the hilus of the glomerulus. The fat in the glomeruli appears first in the periphery, the central portion



being involved only in those glomeruli in which the fat is very abundant.

4. Fatty changes in the glomeruli in acute infections and in glomerulonephritis are apparently the result of direct injury to the cells by some toxic substance in the circulating blood and concentrated in the glomeruli by loss of water by filtration. In nephrosclerosis the anoxia resulting from the ischemia caused by narrowing of the lumen of the afferent artery induces similar effects.

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FATTY CHANGES IN THE GLOMERULI OF THE KIDNEYS*

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In the course of experiments designed to produce glomerulonephritis in dogs, it was found that the glomeruli in many of these animals contained stainable fat. References in the literature to the presence of fat in the glomeruli consist of widely scattered casual observations usually without exact descriptions and are relatively few in number. We have attempted to review the literature, to analyze the occurrence of this condition in our own material from autopsies and from experimental animals, and to arrive at some understanding of its significance.

No adequate data on the frequency of visible fat in the glomeruli are available. Its presence is probably always pathological although Lubarsch suggested that it may sometimes be physiological. Fahr stated that it is associated with lipemia and that in the glomeruli it is an infiltration rather than a degeneration. Lubarsch mentioned, without giving details, fatty changes in the capsular epithelium in 64, in the glomerular epithelium in 16 and "fine dust-like fat" in 232 of 2,720 autopsies, or slightly less than 11.5 per cent. Prym found fat in the glomeruli in 42 out of 211 autopsies, or 19.9 per cent; and Segawa in 46 out of 150 autopsies, or 30.7 per cent. In 28 of Segawa's cases the amount of fat in the glomeruli was described as moderate; in 14, as abundant; and in 4 as very abundant. It is evident from these observations, as well as our own, that fat in the glomeruli is not uncommon in various pathologic conditions. But even with satisfactory stains the fat may be overlooked because of the extreme fineness of the droplets. Diseases in which fatty changes in the glomeruli have been recorded are tabulated as follows:

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Diseases in Which Fat Has Been Found in the Glomeruli

Toxemias and poisonings:

- Acute yellow atrophy of the liver: Mayer (1922).
- Alcoholism: v. Kahlden (1893).
- Bichloride of mercury: Heineke (1909); Bohnenkamp (1922).
- Burns: v. Kahlden (1893).
- Eclampsia: Prym (1910); Segawa (1914); Fahr (1925).
- Phenol poisoning: Peipers (1892).

Metabolic disturbances:

- Beriberi: Segawa (1914).
- Diabetes mellitus: Peipers (1892); Prym (1910); Segawa (1914); Fahr (1920); Lubarsch (1925).
- Exophthalmic goiter: Prym (1914).
- "Arteriosclerosis": Prym (1910); Segawa (1914).

Circulatory disturbances:

- Anemia: Segawa (1914).
- Passive hyperemia: Segawa (1914).
- "Heart disease": Prym (1910).

Other diseases:

- Cirrhosis of the liver: Segawa (1914).
- Jaundice: Prym (1910); Segawa (1914).
- Purpura hemorrhagica: Segawa (1914).
- Malignant tumors: Prym (1910); Segawa (1914).
- Amyloidosis: Tietz (1922); Van Slyke (1930).

Infectious diseases:

- Diphtheria: Nauwerck (1886); v. Kahlden (1893); Segawa (1914); Fahr (1920).
- Dysentery and enteritis: Prym (1910).
- Erysipelas: Segawa (1914).
- Meningitis: Prym (1910); Herzheimer (1916, 1918).
- Peritonitis: Prym (1910); Segawa (1914).
- Pneumonia: Peipers (1892); Gaskell (1911); Segawa (1914).
- Scarlet fever: Löhlein (1904).
- Sepsis: Prym (1910).
- Tuberculosis: v. Kahlden (1891); Prym (1910); Segawa (1914).
- Typhoid fever: v. Kahlden (1893); Gaskell (1911).

Renal diseases:

- Acute glomerulonephritis: Munk (1918); Stern (1924); Volhard (1925); Hückel (1929).
- Chronic glomerulonephritis: Peipers (1892); Löhlein (1905); Prym (1910); Segawa (1914); Volhard (1925); Fahr (1925); Van Slyke (1930); Gray (1933).
- Nephrosclerosis: Prym (1904); Gaskell (1911); Herzheimer (1912, 1916, 1918); Jores (1916); Munk (1918); Tietz (1922); Van Slyke (1930); Gray (1933); Kimmelstiel and Wilson (1936).
- Nephrosis: Löhlein (1918); Munk (1918); Major and Helwig (1925); Fahr (1925); Löwenthal (1927); Bell (1929); Kantrowitz and Klemperer (1931).
- "War nephritis": Herzheimer (1916, 1918); Rochs (1918).

DESCRIPTION OF PLATE

PLATE 119

FIG. 1. Photomicrograph from the kidney of a dog given small doses of diphtheria toxin intravenously, with production of acute glomerulonephritis. The afferent artery is seen to be free of fat. That in the glomerulus itself is concentrated at the hilus and radiates outward in the capillary loops. This type of fatty change is also found in some cases of acute and chronic glomerulonephritis in man. Sudan III stain. $\times 315$.

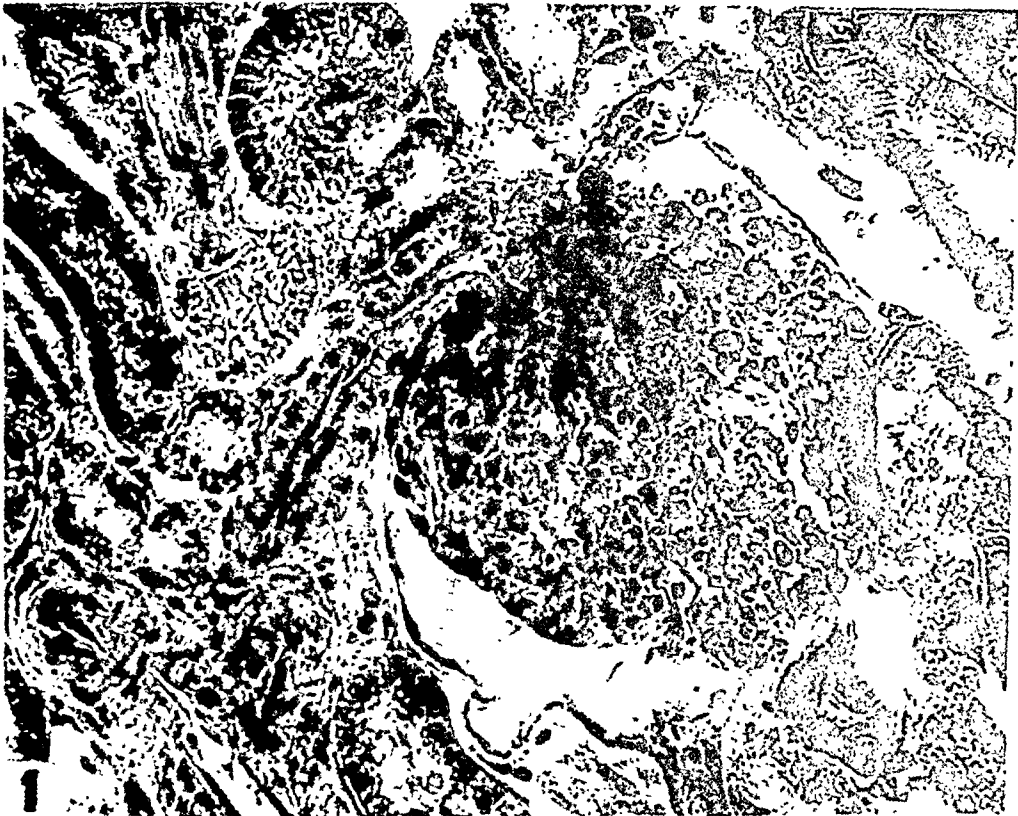
FIG. 2. Photomicrograph showing the type of fatty change seen in the glomeruli in nephrosclerosis. The wall of the afferent artery contains much fat (stained dark) which does not extend into the glomerulus. The fat in the glomerulus is at the periphery. Sudan III stain. $\times 210$.

Of the authors listed, only Jores (1904, 1916), Prym (1904, 1910), Herxheimer (1912) and Segawa (1914) make more than casual comment upon the presence of fat in the glomeruli. The greater part of the references were found by examining the descriptions of microscopic sections of the kidneys in reported cases of the various diseases.

There is no complete agreement as to the location of fat in the glomeruli. Bell, Jores, Aschoff and Kaufmann state that it is in the epithelium; Rochs, Löwenthal and others, that it is in the endothelial cells. Normally the epithelial cells of the glomeruli greatly outnumber the endothelial cells. But in glomerulonephritis, the latter are increased by proliferation. This renders the identification of cells containing fat more difficult. The only cells in which it can be accurately observed are those which line the capsular space.

We have studied sections of kidneys stained with Sudan III from 76 autopsies and from 133 dogs without attempting to differentiate between neutral fats and lipoids. The autopsies were selected largely because they involved diseases of the kidneys. Other cases were chosen as controls, or because fat had been reported in the glomeruli by other observers in the diseases present. Of the autopsies, 30 were from patients suffering from nephrosclerosis and of these 21 (70 per cent) showed fatty changes in the afferent arteries and 12 (40 per cent) in the glomeruli themselves. Among 13 cases of chronic glomerulonephritis, none showed fatty changes in the afferent arteries and 6 had fat, usually in very minute quantities, in the glomeruli. Of 8 cases of acute and subacute glomerulonephritis, 5 showed fat in very fine droplets in the glomeruli. Our miscellaneous group included 1 or more cases each of diabetes mellitus, diphtheria, acute yellow atrophy and cirrhosis of the liver, pneumonia, eclampsia, amyloidosis, abscess of the lung and poisoning with bichloride of mercury and with veronal.

The experiments on dogs were designed to test the theory that a colloidal poison might circulate in the blood in such high dilution as to cause no serious damage to other organs but, as a result of concentration in the glomeruli by loss of water, might injure these structures; and, on the other hand, that a crystalloidal poison might circulate in the blood in sufficiently high dilu-



tion that it could pass through the glomeruli without causing structural or functional damage and yet injure the tubular epithelium as a result of concentration in the tubules by the absorption of water. The total blood volume of each animal was estimated and a known amount of the poison was slowly injected intravenously so that each 100 cc. of the dog's circulating blood would contain a definite concentration in milligrams of the poison or in units of toxin. The results are shown in Table I.

TABLE I
Incidence of Glomerular Fat Following Intravenous Injection of Various Substances

Experiment	Total number of animals	Total number with fat in glomeruli	Percentage
Controls	37	4	18.8
Snake venom	20	6	30.0
Streptococcus toxin	6	2	33.3
Staphylococcus toxin	8	2	25.0
Diphtheria toxin	19	5	27.8
Potassium dichromate	11	4	36.4
Uranium nitrate	22	4	18.2
Bichloride of mercury	10	0	00.0
	133	27	

Of the 37 control animals, most of which had been under ether or nembutal anesthesia for varying periods up to an hour or more, 4, or 10.8 per cent, showed fat in the glomeruli. Two of these were suffering from chronic glomerulonephritis with proliferation of the endothelium of the glomeruli and casts in the tubules. These 2 animals had large quantities of fat in the glomeruli, most concentrated at the hilus. In the other 2 animals, 1 of which had pneumonia, the fat was scanty and in the form of very fine dustlike particles in the walls of the vessels at the hilus.

The incidence of fatty changes in the glomeruli of those animals injected with colloidal poisons (snake venom, and streptococcus, staphylococcus and diphtheria toxins), ranged from 25 to 33.3 per cent. The type of fatty changes in the glomeruli of a dog given minimal doses of diphtheria toxin is illustrated in Figure 1. The fat is concentrated in the region of the hilus of the glomerulus and radiates outward in the walls of the capillary loops. Two of the crystalloidal poisons also produced fatty

changes in the glomeruli, potassium dichromate in 36.4 per cent of the dogs, and uranium nitrate in 18.2 per cent. These include all animals to which these poisons were administered, including those which received more than the optimal dose. On the other hand, none of the dogs poisoned with bichloride of mercury showed fat in the glomeruli although 4 died as a direct result of the action of the poison. Although no very great value is claimed for the statistics of these groups of animals, because they include experiments designed to determine the optimal dose, it may be noted that of 53 dogs injected with colloidal poisons, 15, or 28.3 per cent, showed fatty changes in the glomeruli; while of 43 dogs injected with crystalloidal poisons, only 8, or 18.6 per cent, showed such changes.

DISCUSSION

Fat occurs in the glomeruli in several different forms and locations.

1. In the slighter degrees of this change the fat appears in very fine dustlike droplets in the walls of the capillaries just within the hilus of the glomerulus. The afferent artery is not involved, although we have occasionally seen this form of fat in the juxtaglomerular myoneural apparatus, an observation also made by Goormaghtigh and Handovsky. It is particularly difficult to determine whether the fat in this location is in epithelial or endothelial cells. It is equally difficult to determine whether the fat is in the afferent or in the efferent end of the capillary loops. This is a matter of some importance, as will be pointed out later. This hilar distribution of fat in exceedingly fine particles is characteristic of the fatty changes in the glomeruli in acute infections, such as pneumonia, scarlet fever, peritonitis and glomerulonephritis, and was present in 23 out of the 27 dogs whose glomeruli contained fat.

2. In a more extensive form of fatty change the fat is present in larger droplets, is most concentrated in the region of the glomerular hilus, extends outward along the capillary loops and has much the same distribution as the hyaline thickening of the basement membrane in chronic glomerulonephritis. The afferent arteries are either free from fat or contain only minute quantities and the glomeruli affected are usually well supplied with blood.

DEGENERATION OF THE ADRENAL CORTEX PRODUCED BY GERMANIN*

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The possibility that germanin (Bayer 205) might injure the adrenal glands was suggested by the findings at autopsy of a patient with pemphigus who had been treated with this drug. As previously described¹ the adrenal lesions were of the type often referred to as primary or cytotoxic atrophy, a type frequently associated with symptoms of Addison's disease. The probability that germanin was a factor in the pathogenesis of this patient's adrenal lesions was strengthened by Tomlinson's² report of a similar case. We have failed to find records of similar lesions or of any constant lesions in the adrenal glands of patients with pemphigus who have not been treated with germanin. Preliminary experiments¹ demonstrated that germanin could damage the adrenal cortex, and the present report summarizes the observations on a larger group of animals. In this study we have been less concerned with the general effects of germanin than with ascertaining the type of injury which this therapeutic agent can inflict on the normal adrenal gland.

PROCEDURES

Germanin was administered to 100 guinea pigs, 30 rats, 8 rabbits and 3 dogs, in the form of a 10 per cent solution in freshly boiled distilled water. The drug used was a product of the I. G. Farbenindustrie, marketed for human use. The rats and most of the guinea pigs were injected subcutaneously, the other animals by the intravenous route. All animals were maintained on adequate diets and no adrenal lesions of the types attributed to vitamin deficiency³ were observed in control animals. The main part of this report summarizes the observations on 90 guinea pigs, most of them young males weighing 250 to 350 gm.

In human therapy germanin is administered in courses of variously spaced injections, often after smaller initial doses to detect unusual sensitivity. The maximum single dose considered

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This form of fatty change was especially prominent in 2 of our dogs injected with very small doses of diphtheria toxin in 2 cases of chronic glomerulonephritis.

3. Fat was found in the glomeruli in greater abundance in nephrosclerosis (essential hypertension) than in any other condition. In 70 per cent of our cases of this disease fat was present in the afferent arteries (Fig. 2), and in 40 per cent, also in the glomeruli. We have not observed fat in a glomerulus, the afferent artery of which showed only hyperplastic intimal thickening. The fat is in the form of fine or coarse droplets at the periphery of capillary loops that are almost bloodless. Such glomeruli are frequently lobulated so that the droplets of fat form rounded groups, often so compact that it is difficult to determine whether they are intracellular or free in a necrotic mass. The fat in the afferent artery is usually not continuous with that in the periphery of the glomerulus but is separated by a zone in which fat is absent or present in relatively much smaller quantities. However, occasionally an entire glomerulus contains fat. Prym (1904) has illustrated such a case in which "the capillary loops appeared to consist only of fat." Not infrequently the walls of afferent arteries are thick, hyaline and rich in fat while their corresponding glomeruli are well supplied with blood and wholly free from fat. This type of fatty change is illustrated in Figure 2.

4. The epithelium lining the capsular space may contain visible fat, particularly in cases of acute and chronic glomerulonephritis and in acute infections, such as diphtheria and scarlet fever, and in intoxications. It is usually but not invariably associated with fat in the corresponding glomeruli themselves. The most marked example of this type in our series of cases was in a young woman who died from acute hemorrhagic pancreatitis and had an extreme degree of fatty changes in the liver and in the tubular epithelium of the kidneys. In subacute glomerulonephritis fine fat droplets are often present in the cells of the crescents. In some cases of nephrosclerosis small clumps of fatty cells are seen in the capsular spaces. These are epithelial cells desquamated from the glomeruli either as a result of a superimposed glomerulonephritis (Volhard and Fahr) or of necrobiosis of the capillary loops (Jores).

5. Finally, the hyaline scars which occupy the site of glomeruli

safe for the human adult is 1.0 gm., or 0.02 gm. per Kg. In these experiments the dose of 0.02 gm. per Kg. was employed as the minimum single dose in serial injections imitating courses used in human therapy, while larger single and serial doses were used to ascertain injurious effects. Table I summarizes the dosages employed for guinea pigs. Group A received single injections of amounts from five to twenty times the minimum dose. Groups B, C and D were given serial toxic doses, variously spaced, and in the case of group D, injected intravenously. The courses for groups E, F and G were planned to imitate therapeutic courses and those of groups H and I were similar but prolonged. In some groups the intervals between injections were varied in order to detect cumulative effects, since germanin is known to be eliminated slowly.⁴ Injections were made on alternate days in groups B, E, F, H, and I and at wider intervals in groups C and G. With groups G and I longer rest periods were interposed between series of injections in an attempt to ascertain whether the previously damaged adrenal was more or was less susceptible to injury.

TABLE I
Summary of Dosages and Effects of Germanin

Group	No. of guinea pigs	Doses, gm./Kg.	No. of doses	Intervals (days)	Total gm./Kg.	Total course (days)	No. died	No. with zonal lesions in adrenals
A	12	0.1-0.4	1	0	0.1-0.4	1	4	11
B	12	0.03-0.1	*	2	0.2-0.4	*	7	12
C	8	0.05-0.1	*	8	0.3-0.5	*	2	8
D	6	0.03-0.4	*	2-20	0.09-0.4	*	2	4
E	13	0.02	5	2	0.1	9	0	3
F	13	0.02	10	2	0.2	19	0	5
G	5	0.02	16	2-20	0.32	100†	0	0
H	16	0.02	11-30	2	0.22-0.6	21-59	7	16
I	5	0.02	20-30	2-20	0.3-0.6	59-79†	1	5
Total	90						23	64

* Number of injections and duration of courses varied.

† Rest periods interposed between courses.

The animals that died and surviving animals, killed from 1 to 21 days following the last injection, were autopsied as soon as possible. Those showing pneumonia or other disease were excluded from the study. It was not feasible to free and weigh the adrenals, as handling them caused confusing artefacts. The kidneys and adrenals were fixed and imbedded together in order to standardize the planes of sections and to permit rough com-

cornea. In the third, both posterior extremities were involved. One of these paralyzed animals, in which the corneal opacity did not appear until the eleventh day and the paralysis not until the twelfth day, survived. The other 2 were killed when the paralysis was first observed, on the ninth and tenth days respectively.

Microscopic sections of 3 eyes, presenting well developed opacities of the cornea, showed a keratitis with an infiltration of polymorphonuclear leukocytes in the cornea and an extensive infiltration, composed of mononuclear cells and polymorphonuclear leukocytes, in the adjacent conjunctiva and the ciliary bodies. In the section of 1 eye the external half of the cornea and the adjacent conjunctiva was completely necrotic. Sections from the eye removed at the time of the first appearance of opacity showed only a slight inflammatory reaction, limited to the edge of the cornea and to the immediately adjacent conjunctiva. The epithelium covering the cornea of the latter eye was still intact, and at the edge of the cornea the epithelial cells were enlarged and undergoing proliferation. Several mitotic figures were seen. However, no intranuclear inclusions were observed.

In the brain of the rabbit which died on the fifth day after intracerebral inoculation a most intense inflammatory and necrotizing process was seen. The meninges showed an extensive exudate of lymphocytes, mononuclear cells, and polymorphonuclear leukocytes which was partially necrotic. In the outer part of the cortex of the cerebrum, at times continuous with the meninges, there were large areas of early necrosis, in and about which there was an infiltration of many polymorphonuclear leukocytes. In many nerve cells adjacent to these areas of necrosis, conspicuous large acidophilic inclusions were seen within the nuclei. These inclusions varied considerably in size, some completely filling the nucleus except for a deep-staining margin of chromatin. The staining of the inclusions with phloxine-methylene blue varied from light pink to lilac. In sections from the other 3 brains, 1 of a rabbit inoculated intracerebrally and 2 of animals inoculated on the scarified cornea, a well preserved meningeal exudate of lymphocytes and mononuclear cells was seen. In addition there were perivascular infiltrations of lymphocytes, focal accumulations of irregularly shaped mononuclear cells resembling those seen in the human brain, and occasional small, localized areas of

degenerating brain tissue, usually adjacent to the meninges. Nerve cells containing intranuclear acidophilic inclusions occurred, but these were far less numerous than in the brain of the rabbit dying on the fifth day.

Rats. Of 12 rats inoculated intracerebrally with 0.06 to 0.08 cc. of the supernatant fluid of a 10 per cent emulsion of infected mouse brain, 5 died 3 to 5 days following inoculation. The brains of 3 of these were examined microscopically. There occurred an infiltration of mononuclear cells in the meninges similar to that seen in the other species but with little necrosis of the exudate. Within the brain substance were seen changes comparable to those in the rabbit brains, but less severe. Intranuclear inclusions, though not numerous, were found in the nerve cells, always in areas where an inflammatory reaction was present.

Monkeys. Three rhesus monkeys, inoculated intracerebrally, remained well.

Chick Embryo. The virus was inoculated on the chorio-allantoic membrane of the developing chick embryo. Large, discrete, opaque foci were observed when the membranes were examined on the second and third days following inoculation. Microscopically, the membranes showed a marked proliferation of the ectodermal layer of cells. Many of these cells contained intranuclear acidophilic bodies, and the lesions resembled, in general, those described for the virus of herpes simplex.

NEUTRALIZATION OF THE VIRUS

Neutralization tests were carried out in mice with the R. T. virus and with a known herpes simplex virus (Rockefeller Institute H. F. strain). The serums used were an immune serum prepared by inoculating rabbits with the R. T. virus and two serums prepared by immunizing chickens (one with a known herpes virus (H. F. strain), and the other with a herpes virus modified by passage in the chicken embryo*). The technic used in the neutralization test was as follows: A 10 per cent suspension of virus was prepared by grinding infected mouse brains with a requisite amount of broth. After light centrifugation, the supernatant fluid was removed and serial tenfold dilutions in broth were made. Virus

* We are indebted to Miss Katherine Anderson, of the Department of Pathology, Vanderbilt University, for supplying us with the immune chicken serums.

parisons of size. Usually sections of the liver and heart, and sometimes of other tissues, were prepared. Sections of kidneys, liver and heart were examined for fat.

RESULTS

The Effects of Single Toxic Doses of Germanin (Table II)

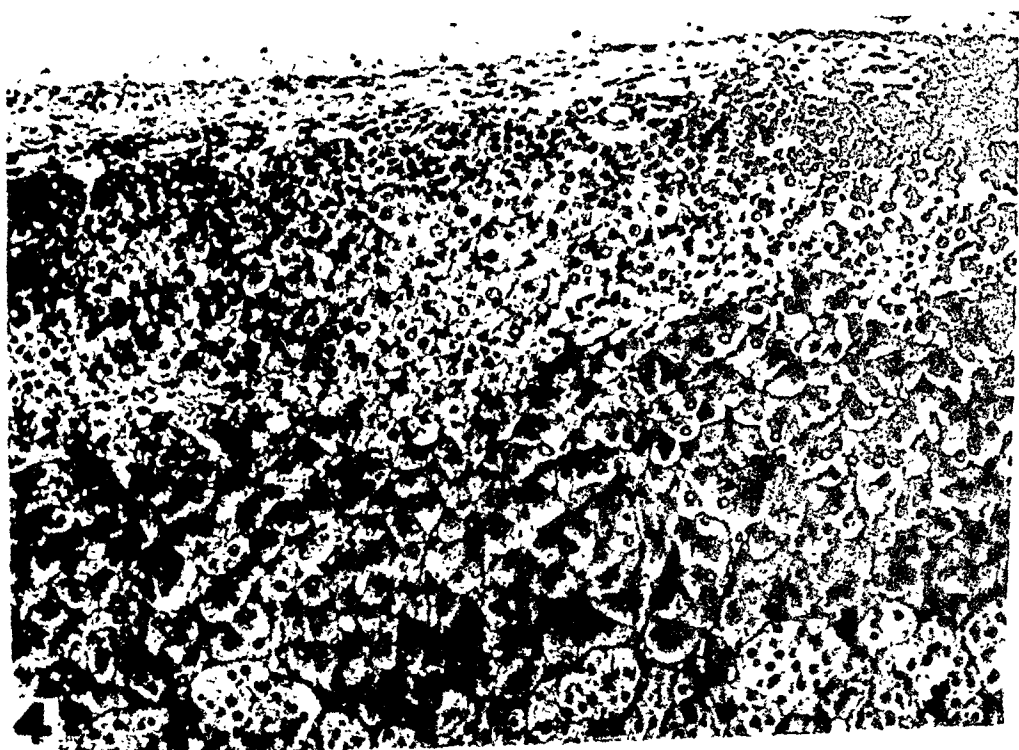
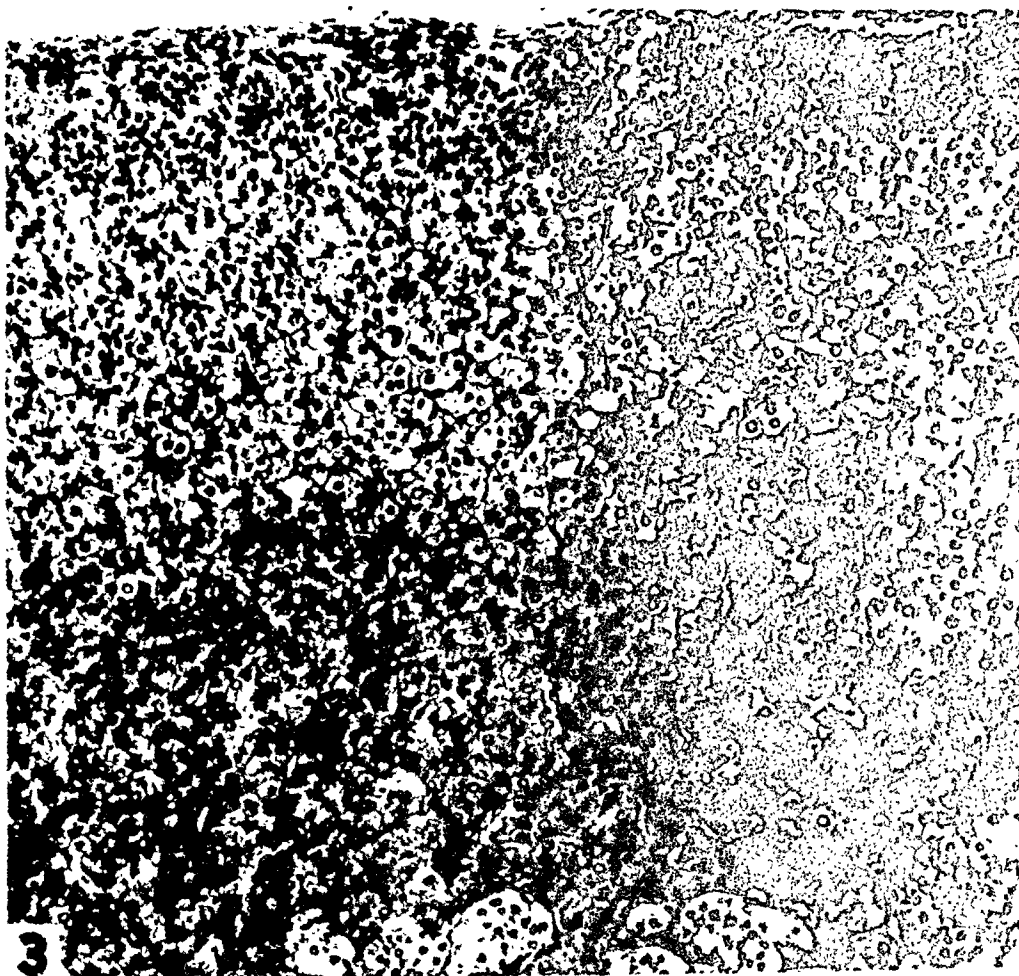
Adrenal cortical lesions were found in 11 of 12 guinea pigs given single injections of from 0.1 to 0.4 gm. of germanin per Kg. These were constantly located in the outer part of the fasciculate zone, sometimes encroaching on the glomerular zone. The type characterized as an *acute degenerative band* (Fig. 1) was seen in 4 animals, all dying early after doses of 0.3 and 0.4 gm. per Kg., the established lethal dose for the guinea pig.⁵ These bands, one-third to one-half the width of the cortex, were separated from the capsule by only a few groups of small dark cells. They showed completely disorganized trabeculae, degenerating cells with pyknotic or fading nuclei, scattered epithelial cells with rounded borders, and neutrophilic leukocytes. Besides these

TABLE II
Effect on the Adrenals of Single Toxic Doses of Germanin

Guinea pig No.	Dose, gm./Kg.	Died or killed	Day	Adrenal zonal changes	Maximum No. of mitoses per high power field
1	0.4	Died	5	Acute degenerative bands	0
2	0.4	Died	5	Acute degenerative bands	0
3	0.3	Died	5	Acute degenerative bands	0
4	0.3	Died	6	Acute degenerative bands	0
5	0.3	Killed	8	Broad reactive bands	3
6	0.3	Killed	14	Broad reactive bands; more reparative activity	8
7	0.2	Killed	8	Like No. 5; narrower	3
8	0.2	Killed	14	Broad reactive bands; more reparative activity	8
9	0.2	Killed	21	Advanced repair	20
10	0.1	Killed	8	Narrow, but like Nos. 5 and 7	3
11	0.1	Killed	14	Advanced repair	3
12	0.1	Killed	21	None	2

sharply demarcated bands, many scattered degenerating cells were seen throughout the cortex.

Lesions characterized as *reactive bands* were seen in animals which survived doses of 0.1 to 0.3 gm. per Kg. They contained remnants of degenerating cells, swollen endothelial cells and macrophages, a few neutrophils, lymphocytes and eosinophils,



and sparse large eosinophilic epithelial cells. Mitotic figures, a few of them in endothelial cells but mostly in epithelial cells, were found throughout the cortex. They were especially numerous in and near the bands. In the older lesions large and sometimes multinucleated epithelial cells were invading the bands from both sides (Fig. 2).

The absence of changes in the adrenals of guinea pig No. 12 should be noted. Since a dose of this size invariably produced zonal lesions it seems likely that the interval of 3 weeks had sufficed for complete healing.

The Effects of Multiple Toxic Doses of Germanin

Twenty guinea pigs were given series of injections with single doses of 0.03, 0.05 or 0.1 gm. per Kg. each. Twelve (group B) were treated intensively, with injections on alternate days, while 8 (group C) were injected 8 days apart. Eleven animals of group B died or were killed because they were moribund, while only 2 of group C died. The difference is especially significant since most of the members of group C were given larger single and total doses.

The character of the zonal adrenal lesions, present in all, varied considerably. Those of 4 animals, dying after intensive series of 3 or 4 injections of 0.1 gm. per Kg. each, were *acute degenerative bands*. These were indistinguishable from those resulting from single injections of 0.3 and 0.4 gm. per Kg. The adrenals of animals treated intensively but with smaller doses showed *mixed degenerative-reactive bands*. An interesting feature was the presence in and near these bands of large cells with bizarre nuclei, some of them certainly abnormal and degenerating mitotic figures. The appearance suggested that acute injury had been superimposed on a phase of repair. Some members of group C had similar lesions, but others presented a different type, the *collapsed band*, which was usually observed in a small gland. These bands were narrow, devoid of epithelium and made up of stromal elements with wide capillaries lined by numerous but flat endothelial cells. Here and there in bands a few lymphocytes and macrophages filled with yellow (lipochrome?) pigment were present. There was no fibrous hyperplasia and the whole appearance suggested the condensation of a previously wider

zone in which repair had been arrested. However, there was some reparative activity at the borders, save in the animals that died.

The Effects of Multiple Small Doses of Germanin

The courses of the drug given animals of groups E, F and G paralleled some of those reported as having been used in human therapy. There were definite zonal changes of the mixed type and some thinning of the cortex in 3 animals given 5 doses of 0.02 gm. per Kg. and in 5 animals given 10 similar injections. Slight degenerative changes were seen in the adrenals of other members of both groups. Figure 3 shows the adrenal lesion of a member of group F. No lesions were observed in the guinea pigs of group G, given 16 widely spaced doses of 0.02 gm. per Kg. each. There were no fatalities in these groups.

The members of groups H and I received from 11 to 30 injections of 0.02 gm. per Kg.; in the case of Group I, with a 20-day rest period after the 15th dose. The 8 fatalities were not determined by total dosage and some followed courses only a little more prolonged than those of group F (see Table III). Adrenal lesions were present in all and every animal given more than 15 doses had collapsed bands, usually in glands which were small. In the smallest glands seen after the longer courses the surviving cortical cells were large and eosinophilic, with large vesicular nuclei (Fig. 4). They resembled the islands of large cells sometimes seen in human adrenal glands showing cytotoxic atrophy. Regenerative activity was noted at the margins of the bands save in the adrenals of some of the animals that died.

Table III presents the data for 13 guinea pigs of groups E, F and H, arranged in order of increasing total dosage. These animals were comparable as to size, sex and survival. All died or were killed 2 days after the final injection.

Evidence for the Cumulative Action of Germanin

The differences in mortality and in character of adrenal lesions in groups B and C are almost certainly attributable to the cumulative action in the intensively treated group B and the longer time for the elimination of the drug between doses in group C. Other animals, paired as to dosage and differing only as to the spacing of injections, showed similar differences. For instance,

ACUTE LOCAL ANAPHYLACTIC INFLAMMATION OF THE LUNGS*

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The phenomenon of acute local anaphylactic inflammation is not clearly understood, either as to its mechanism of development or its relationship to immunity. Its specificity is generally agreed upon, but the type and source of antibodies are still uncertain. Although there is evidence that the local anaphylactic reaction results from the union of antigen with precipitins in the blood and tissues, some investigators attribute it to a specifically changed reaction-capacity of tissues and suggest that this may be entirely independent of demonstrable antibodies, either in the blood or tissues. Some also regard the entire process as harmful, to be eliminated whenever possible, whereas others consider it as ordinarily beneficent, although occasionally injurious.

A part of the uncertainty relating to the entire problem has come from the use of complex antigens, particularly bacterial cultures, vaccines, or animal proteins containing several antigenic components. Furthermore, these materials have usually been injected into solid tissues, such as the skin, joints, kidneys, heart and aorta—organs in which interstitial, parenchymal and vascular lesions cannot be readily differentiated. Inasmuch as there is considerable evidence that the primary reaction of local anaphylaxis is vascular, it is desirable, in order that the primary lesions may not be obscured by secondary effects, to study its development in a vascular organ containing a minimal quantity of interstitial and parenchymal tissue. The lungs of rabbits, because of their vascularity, seem well suited for such a study, especially since they play such an important part in the development of the anaphylactic reaction in this species. They afford an excellent opportunity, furthermore, for the nontraumatic introduction of the antigen into the air passages where the reaction can develop as a surface phenomenon on a "blood-tissue"

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guinea pig No. 20 (Table III) received 16 injections of 0.02 gm. per Kg. on alternate days, or in 31 days, while several animals of group G received the same dosage in 100 days. The first animal died and its adrenals had collapsed bands with little regeneration, while the other animals had normal adrenal glands.

TABLE III
*Adrenal Lesions After Serial Small Doses of Germanin**

Guinea pig No.	No. of doses	In days	Died or killed	Cortical zonal lesions in the adrenal gland†
13	5	9	Killed	Narrow band with a few degenerating cells
14	10	19	Killed	Band with mixture of degeneration and repair
15	11	21	Died	Similar; less repair
16	12	23	Died	Collapsed band; slight repair
17	14	27	Died	Mixed band with regions of collapse
18	15	29	Killed	Collapsed band; some repair
19	15	29	Killed	Collapsed band; good repair
20	16	31	Died	Collapsed band; no repair
21	20	39	Died	Collapsed band; no repair
22	20	39	Killed	Collapsed band; slight repair
23	25	49	Killed	Similar; fair repair
24	26	51	Died	Collapsed band with marked acute degeneration in adjacent cortex
25	30	59	Killed	Collapsed band; slight repair

* Single doses of 0.02 gm. per Kg. were injected subcutaneously on alternate days. All animals were comparable as to initial weight and sex. All died or were killed 1 or 2 days after the final injection.

† With few exceptions these adrenal glands were small in comparison to the size of the animal.

Character of Adrenal "Atrophy" Caused by Germanin

Decrease in size of the adrenal glands was seen mainly in animals given repeated small injections and was especially marked in those receiving 20 or more doses. The process certainly was not a simple atrophy but resulted from the disappearance of parenchymatous cells, collapse of the less damaged stroma and lagging repair. A striking similarity to the processes in so-called acute yellow atrophy of the liver was noted by Wells, Humphreys and Work.¹ In both cases, if the process is to be characterized as "atrophy," the qualifying term of "cytotoxic" is warranted.

Miscellaneous Toxic Effects of Germanin on Guinea Pigs

Losses of weight and strength were the most obvious general manifestations, and after prolonged courses losses of from 20 to 40 per cent of the initial weight were noted. Since most of the animals were young, even the lesser losses of weight were significant.

barrier; *viz.*, the membrane which lines the alveolus and separates it from the lumen of the intra-alveolar capillary.

Only a few investigators have made histological studies of anaphylactic inflammation in the lungs. Earlier workers ^{1,2} observed the development of pneumonitis in guinea pigs sensitized to a foreign serum following inhalation of a spray of the serum; Schlecht and Schwenker ³ described pulmonary hemorrhages and foci of acute alveolitis appearing as early as 6 hours after inhalation. Opie ⁴ injected 0.2 cc. of horse serum through the thoracic wall into the lung of an immunized rabbit and found that it caused "localized consolidation with leucocytes and edema surrounding a central focus of necrosis," whereas, "the same antigen injected into a normal rabbit was absorbed from the lung with no noteworthy change." Fried ⁵ injected horse serum directly into the trachea of normal and of sensitized rabbits and noted in the latter an intensified inflammation of the lungs, characterized by edema, hemorrhage, leukocytic infiltration, deposition of fibrin, consolidation and necrosis.

The following experiments were performed with the object of studying histologically the pulmonary lesions which might develop in normal and protein-sensitized rabbits following the entrance of a solution of protein into the lungs.

MATERIALS AND METHODS

Rabbits from a source carefully guarded against snuffles were made hypersensitive by several subcutaneous injections, at intervals of from 5 to 7 days, of a solution of dried egg white or of crystalline egg albumin (crystallized three times). The latter substance is a relatively homogeneous purified antigen which is but slightly irritative to normal tissues. For some of the tests a solution of partially purified egg albumin, representing the albumin fraction after the first precipitation with ammonium sulfate, was used. This is referred to as albumin precipitate. The rabbits, when adequately sensitized, as shown by an intradermal injection of 0.1 cc. of a 2 per cent solution of the protein, were paired with normal rabbits and a solution of either crystalline egg albumin or of the albumin precipitate was instilled simultaneously into their nostrils. (It is well known that light fluids, when introduced into the nostrils of unanesthetized rabbits, flow

In general, germanin is not to be classed as a steatogenic poison nor one causing fatty degeneration, and fatty changes were usually insignificant. There were, however, a few large (800 to 1150 gm.) animals, all given toxic doses. While the other effects were similar, there was one striking difference. The kidneys, livers and hearts of these mature guinea pigs showed profound fatty changes. Since young animals had been given similar doses without showing fatty changes, the difference in age seemed to be the only explanation.

Clinical observations and previous experimental studies have stressed the injurious effects of germanin on the kidneys. Renal lesions were common in this series. Large doses caused profound tubular degeneration with hydropic changes and sloughing of epithelium, and small serial doses usually caused some tubular damage. However, the kidneys frequently showed marked regenerative hyperplasia. In some animals with adrenal lesions they were essentially normal. Either they were less susceptible to injury or had recovered more rapidly.

Changes in other organs were not conspicuous. Toxic doses caused some degenerative changes in the liver, but these were not spectacular. In a few cases the lungs showed edema or small hemorrhages. The bone marrow, examined only a few times, was not unusual. The same was true of the spleen, pancreas, thyroid and lymph nodes. The brain was not examined.

Effects of Germanin on Rats, Rabbits and Dogs

Too few animals were studied to permit more than general observations. Adrenal lesions similar to those of guinea pigs were observed in both rats and rabbits. After toxic doses, rats frequently had petechiae in the skin and in mucous and serous membranes, and large subcutaneous hemorrhages at the sites of injections. No such hemorrhagic tendency was observed in rabbits and guinea pigs. No lesions were observed in 3 dogs, all of them young animals, given small series of injections with minimum doses.

SUMMARY AND DISCUSSION

This study confirmed previous observations on the toxic and lethal effects of germanin, its cumulative action and the capacity of toxic doses to injure the kidneys. It established the fact that

readily down the trachea into the lungs. This obviates the necessity for intratracheal injection or for direct injection through the thoracic wall with the complicating elements of surgical trauma, hemorrhage, shock or interference by anesthesia.) At varying intervals the animals were killed by air embolism and the lungs fixed *in situ* with a Zenker-formaldehyde solution. Celloidin sections were prepared and stained with hematoxylin and eosin. In some instances rabbits were sensitized passively by the intravenous or intraperitoneal injection of serum from rabbits hypersensitive to crystalline egg albumin and the effects following instillation of the solution of protein into their lungs observed. In some of the animals, also, the precipitative titers, as determined by the collodion particle agglutination method,⁶ were determined at the time of instillation of the protein solution.

RESULTS

Findings in Normal Rabbits

Sixteen normal rabbits were used as controls and their lungs examined from 4 to 48 hours after intranasal instillation of the protein solutions. Eleven of the animals were killed 24 hours after the instillation. The effects of the entrance of the egg albumin into their lungs were for the most part slight. Despite the fact that several blocks of tissue were taken from each lung, and that an effort was made to select the most abnormal looking areas, most of the sections showed no significant change. An occasional slight area of acute alveolitis was seen in a few instances, with an associated minimal edema. Acute hemorrhage was never seen and the perivascular lymphatics usually appeared normal. There was no phlebitis or arteritis and no thrombosis. Almost all sections were described as normal and inflammation, when present, was minimal. Crystalline egg albumin or a solution of dried egg white, therefore, may be regarded as practically nontoxic in the amounts and concentrations that entered the lungs of these normal rabbits.

Findings in Rabbits Sensitized Against Egg Albumin

Nineteen hypersensitive rabbits were treated simultaneously with the controls. The effect of the entrance of the protein solutions into their lungs, however, was strikingly different, being

germanin has a selective action in damaging the adrenal cortex, equal to or surpassing its ability to injure renal tubular epithelium. It is impossible in most cases to estimate the relative importance of renal and adrenal damage in causing death of the animals. However, in a few instances, animals that died had zonal lesions of the adrenals and practically normal kidneys.

Even more important than the fact that large doses of germanin may cause extensive destruction of the adrenal cortex is the fact that small doses, comparable to those used in man, may cause similar though less extensive lesions. It is significant that the zone where the damage occurs is the zone of the cortex where growth is normally most active. This undoubtedly was a factor in the decrease in size or "atrophy" noted after many small doses. However, with normal animals and with the dosages used, the destruction of the cortex was never complete, and in surviving animals the capacity to regenerate was not completely lost. There was no evidence that sensitization to germanin played a part in causing the adrenal lesions of these animals.

As to cytotoxic atrophy of the adrenal glands in man, it seems unlikely that germanin can be responsible for more than rare cases. The drug is little used save in the treatment of African sleeping sickness, pemphigus and a few other cutaneous diseases. Talbott, Lever and Consolazio⁶ questioned the importance of the rôle of germanin in the human cases cited,^{1,2} since they found chemical changes in the blood of patients with pemphigus which pointed to adrenal insufficiency. However, their observations may have other significance. It is not impossible that a functionally abnormal adrenal gland might have an increased susceptibility to a toxic agent which acts selectively to injure the adrenals. Also, there is still the possibility that drug sensitization may play a part, a possibility not excluded by the failure to demonstrate sensitization in one animal species. It should be noted that lesions resembling cytotoxic atrophy have not been reported in patients with pemphigus who have not been treated with germanin. In fact there seem to be no constant or specific adrenal lesions.

The demonstration that one agent used in the therapy of human disease can consistently injure the adrenal cortex of experimental animals and that it almost certainly has caused cytotoxic atrophy of the adrenal glands of human beings is important in

characterized by acute edema, alveolitis, bronchitis and pneumonic consolidation (Figs. 1 and 2). The severity of the reaction depended essentially upon the degree of sensitivity of the animals, the amount of material which apparently entered the lungs and the length of time after its instillation. The lymph flow was markedly increased (Fig. 3) and at times hemorrhage occurred, both into lymphatic spaces and into alveoli (Fig. 4). Acute arteritis and phlebitis were present in some animals, with, in some instances, mural thrombosis in small arteries and veins (Fig. 6). Infarction also occasionally developed. The effect was as if the nontoxic solution of protein had become toxic, at times extremely so. Thus, the primary and outstanding effect was upon blood vessels and was made evident particularly by increased capillary permeability. Only when the effect was more intense did arteritis, phlebitis, infarction and marked pneumonic consolidation occur.

Findings in Rabbits Passively Sensitized

An attempt was made to ascertain whether the pulmonary lesions which appeared so strikingly in actively sensitized animals could be demonstrated also in those passively sensitized. Sera from several rabbits which were strongly hypersensitive to crystalline egg albumin were pooled and 50 cc. were injected intravenously into two normal rabbits (P₃ and P₄). At the end of 24 hours the animals were found to be definitely hypersensitive to egg albumin and samples of their sera were taken. A solution of precipitated egg albumin was then instilled intranasally and, at the end of 24 hours, a second sample of blood was taken, the animals were sacrificed and their lungs prepared for histological study. The two samples of sera, with serum from a normal animal as a control, were tested against collodion particles to which crystalline egg albumin had been adsorbed. The precipitative titers of both hypersensitive rabbits showed a decline from 1:1920 to 1:120 as a consequence of the intranasal instillation of the albumin and its combination with antibody in the lungs. Sections of the latter (Figs. 7 and 8) showed acute edema, deposition of fibrin within alveoli, early acute alveolitis and hemorrhage, with erythrocytes present in dilated perivascular lymphatic spaces and alveoli.

itself. Even more important, however, is the fact that it emphasizes the necessity for systematic morphologic studies in this period of increasing use of chemotherapy. It proves that it is not enough to examine the large viscera. Germanin has been in use for 20 years, yet we have failed to find mention of examination of the adrenal glands save by gross inspection.

CONCLUSIONS

Germanin in toxic doses consistently produces zonal degeneration of the adrenal cortex of small laboratory animals.

Small serial doses of germanin, comparable to those in therapeutic use in man, may produce similar but less intense adrenal cortical lesions.

It is probable that germanin has occasionally caused cytotoxic atrophy of the adrenal glands in patients with pemphigus.

It is possible that other therapeutic agents may have a similar selective action and a capacity to injure the adrenal cortex.

NOTE: This investigation was carried out under the direction of H. Gideon Wells, to whom we are grateful for advice and criticism.

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Two additional rabbits, with two controls, were similarly treated (P6 and P7) and sacrificed at the end of 48 hours. Sections from the lungs of the normal rabbits showed nothing unusual in one and only a few tiny areas of acute alveolitis in the other. In the sensitized animals, however, the pulmonary inflammation was intense, being characterized by marked acute focal alveolitis, dilation of the perivascular lymphatic spaces and accumulations of mononuclear cells and masses of fibrin in the alveolar spaces.

Inasmuch as passive sensitization in the preceding experiments was accomplished by way of the blood stream, an attempt was made to produce it directly within the alveolar spaces. Fox⁷ has shown that immune sera, when introduced into the lungs by way of the bronchi, are absorbed but slowly into the blood stream. This is due, presumably, to the slow diffusibility of the larger globulin molecules. Ten cc. of serum from a rabbit hypersensitive to crystalline egg albumin were instilled into the nostrils of a normal rabbit. Twenty minutes later 2 cc. of a 4.4 per cent solution of albumin precipitate were then instilled intranasally. The animal was sacrificed 30 hours later. Sections showed a more marked acute edema and focal alveolitis than were seen in rabbits P3 and P4, with many polymorphonuclear leukocytes centered around eosinophilic masses (precipitate?). This finding would suggest an antigen-antibody reaction occurring largely on or within the alveolar walls, with the increased capillary permeability and acute inflammation ensuing.

It is obvious, therefore, that the local anaphylactic reaction is essentially the same, whether it occurs in rabbits actively or passively sensitized. This must mean that the determining element in the reaction is humoral, presumably the anaphylactic antibody and not primarily a changed reaction-capacity of the tissues.

DISCUSSION

These findings corroborate those of others that, whether in general or local anaphylaxis, much of the reaction is vascular and manifests itself as an increased capillary permeability as the result of specific injury to endothelium. Moon⁸ has recently called attention to the striking similarity between the circulatory

DESCRIPTION OF PLATES

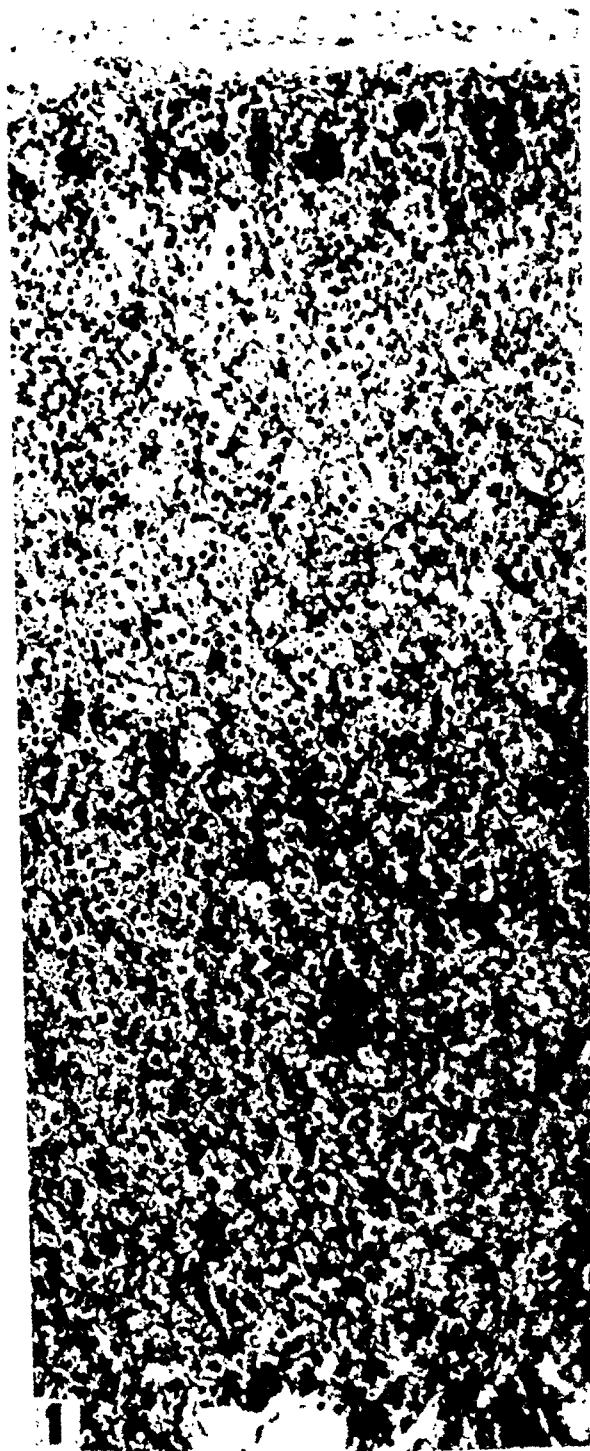
PLATE 120

FIG. 1. Acute zonal degeneration of the adrenal cortex of a guinea pig dying 1 day after a course of 4 injections of 0.1 gm. of germanin per Kg. Similar lesions were produced by single doses of 0.3 and 0.4 gm. per Kg. The band involves nearly one-half of the cortex and encroaches upon the glomerular as well as upon the fasciculate zone. There are many degenerating cells throughout the cortex and polymorphonuclear leukocytes invade the band. $\times 140$.

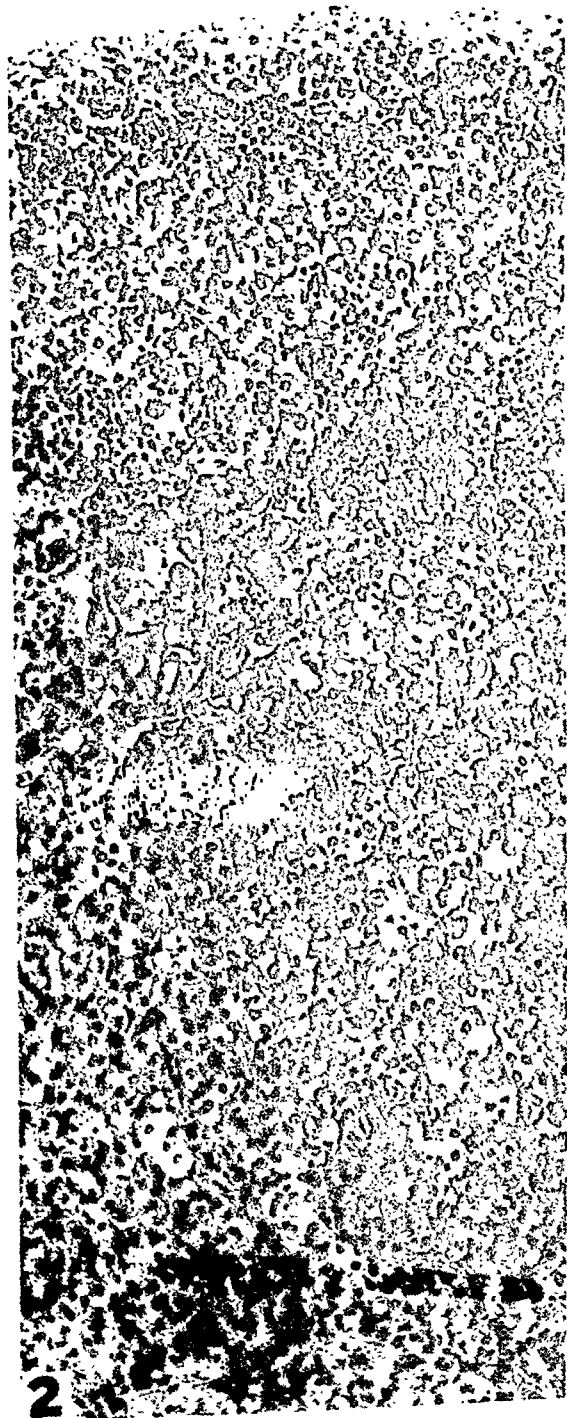
FIG. 2. Repair of zone of degeneration in the adrenal of a guinea pig killed 21 days after a single injection of germanin of 0.2 gm. per Kg. There are many mitotic figures. These are most numerous near and in the band. $\times 140$.

disturbances seen in anaphylactic shock and in experimental or clinical traumatic shock and has emphasized the point that in anaphylaxis "the capillary endothelium is a chief point of injury." The injury occurs, presumably as a result of the union of antigen and antibody within the sensitized endothelium. Manwaring, Chilcote and Hosepian,⁹ and Petersen and Levinson¹⁰ have shown that in anaphylactic shock the endothelium may be damaged to the point of allowing erythrocytes to pass through. Zander¹¹ has shown recently, by means of the application of a partial vacuum to the skin of rabbits, that allergic inflammation leads to an increased tendency to capillary hemorrhage or, as he calls it, an increased capillary fragility. Direct evidence of the effect of an antigen-antibody reaction upon vascular permeability is furnished in the experiments of Abell and Schenck.¹² These observers studied the action of horse serum introduced into the moat of an ear chamber in rabbits sensitized to horse serum. They observed contraction of arterioles, with stoppage of circulation, an increased tendency of leukocytes to adhere to the endothelium and passage of leukocytes in large numbers through the walls of capillaries and venules. Leukocytes, at times, also formed clumps large enough to cause embolic blockage in capillaries and venules. With repeated introduction of horse serum into the moat, even extravasation of erythrocytes and endothelial destruction occurred. Rich and Follis¹³ more recently have concluded that, as a result of studies of the Arthus phenomenon in the corneas of sensitized rabbits, "the sensitivity that determines necrosis appears to be limited to the blood vessels, and especially to the endothelium" whereas "the cells of the tissues at large are not themselves sensitized."

The exact cause of the increased capillary permeability is uncertain. Lowered oxygen tension with resulting asphyxia of the vessel wall is usually considered of great importance as a cause of such a change. It is possible that this may occur also when antigen and precipitin combine on or within endothelial cells. The interference with cellular respiration might be manifested quickly by the increased permeability which is so conspicuous a feature of the anaphylactic reaction. A final answer cannot be given, however, until such intracellular precipitation can be demonstrated in living endothelium.



Humphreys and Donaldson



Adrenal Lesions from Germanin

SUMMARY

Rabbits made hypersensitive (actively or passively) to purified egg albumin, and normal controls, were given simultaneous intranasal instillations of a solution of purified egg albumin. They were sacrificed at varying intervals, the lungs were fixed *in situ* in a Zenker-formaldehyde solution and sections were stained with hematoxylin and eosin.

In the normal animals pulmonary inflammation was minimal and frequently indiscernible. In the hypersensitive rabbits, however, the entrance of egg albumin into the lungs engendered the development of acute pneumonitis, characterized by edema, alveolitis, bronchitis and pneumonic consolidation. The perivascular lymphatics were dilated and contained many erythrocytes. Acute arteritis and phlebitis occurred at times and mural thrombosis was occasionally seen. The findings were essentially identical, whether the rabbits were actively or passively sensitized.

The experiments indicate, therefore, that the primary effect of the antigen-antibody reaction in the lungs was an increased capillary permeability, followed later by severer vascular injury. They show, furthermore, that the effect is due to a humoral element, presumably the anaphylactic antibody, rather than to a changed reaction-capacity of the tissues. The intensity of the reaction depends, apparently, upon the varying intensities of the antigen-antibody union.

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PLATE 121

FIG. 3. Mixed degenerative-reparative changes in the adrenal gland of a guinea pig killed 2 days after a course of 10 injections of germanin, of 0.02 gm. per Kg. each, given on alternate days. The cortex appears thinner than it is normally. $\times 140$.

FIG. 4. Collapsed cell-poor band with marginal regeneration in the adrenal of a guinea pig killed 8 days after a course of 30 injections of germanin of 0.02 gm. per Kg. each, given on alternate days. The thinness of the cortex is not an artefact as the plane of the section is the same as in Figures 1 to 3, and this animal weighed 100 gm. more than any of the other three guinea pigs. $\times 140$.

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DESCRIPTION OF PLATES

PLATE 122

- FIG. 1. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 10 per cent crystalline egg albumin. Acute edema and alveolitis are present. $\times 125$.
- FIG. 2. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 5 per cent crystalline egg albumin. Taken from an area showing acute bronchitis and bronchopneumonia. $\times 125$.
- FIG. 3. From the lung of a rabbit actively sensitized against crystalline egg albumin 8 hours after intranasal instillation of crystalline egg albumin, showing marked dilation of a perivascular lymphatic space. $\times 130$.
- FIG. 4. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of crystalline egg albumin. The increased vascular permeability is shown by the presence of erythrocytes in the perivascular lymphatic space. $\times 290$.

dilutions of 10^{-2} , 10^{-3} , and 10^{-4} were used with undiluted immune serum. To 0.2 cc. of each virus dilution was added 0.4 cc. of serum. The mixtures were incubated for 2 hours at 37° C. and then injected intracerebrally in 0.03 cc. amounts into groups of 4 Swiss mice. The animals were watched daily during an observation period of 3 weeks for evidence of infection. Table I shows

TABLE I

Cross Neutralization Tests with Herpes Simplex Virus (Rockefeller Institute H. F. Strain) and with the R. T. Virus

Serum	Virus	Duration of life of test mice		
		Dilution of virus used in serum mixtures		
		10^{-3} days	10^{-3} days	10^{-4} days
Rabbit, immune to R. T. virus	R. T. virus	1, 12, S*, S	S, S, S, S	S, S, S, S
Rabbit, normal	R. T. virus	1, 4, 5, 5	5, 6, 6, 7	11, 13, 15, S
Rabbit, immune to R. T. virus	Herpes simplex	5, S, S, S	S, S, S, S	S, S, S, S
Rabbit, normal	Herpes simplex	4, 5, 5, 6	4, 6, 7, S	11, 19, S, S
Chicken, normal	R. T. virus	4, 4, 4, 6	7, 7, 10, 12	9, 11, S, S
Chicken, herpes (H. F.) immune	R. T. virus	5, 6, 6, 11	11, 12, S, S	13, S, S, S
Chicken, herpes (modified) immune	R. T. virus	6, 7, 10, S	S, S, S, S	S, S, S, S
Chicken, normal	Herpes simplex	3, 3, 3, 4	6, 7, 7, S	5, S, S, S
Chicken, herpes (H. F.) immune	Herpes simplex	6, 8, 9, S	S, S, S, S	5, S, S, S
Chicken, herpes (modified) immune	Herpes simplex	6, 7, 7, S	S, S, S, S	10, S, S, S

* S = Mouse remained well 21 days.

the results of the cross neutralization tests between a strain (H. F.) of known herpes simplex virus and the R. T. virus. It is readily apparent that immune serum to each virus was capable of neutralizing both viruses to the same extent and that the two viruses are immunologically identical.

DISCUSSION AND SUMMARY

A child, 4 weeks old, was brought to the hospital because of irritability, refusal to nurse, and twitchings of the left side of the body, and died on the fifth hospital day after a progressive accentuation of the cerebral symptoms. From the brain tissue taken at autopsy, a virus was isolated in mice which has been identified as that of herpes simplex.

During life the child's spinal fluid was sterile and no micro-organisms were cultivated in dextrose infusion broth inoculated with an emulsion of the brain tissue. Furthermore, continued attempts to cultivate bacteria from the brains of mice, which died following the inoculation of infectious material originally derived from the human brain, have yielded negative results. The fact that we have been unable to show that the virus will pass through either Berkefeld N or V filters does not militate against its identification as herpes simplex, which is known to be filtrable only with difficulty.

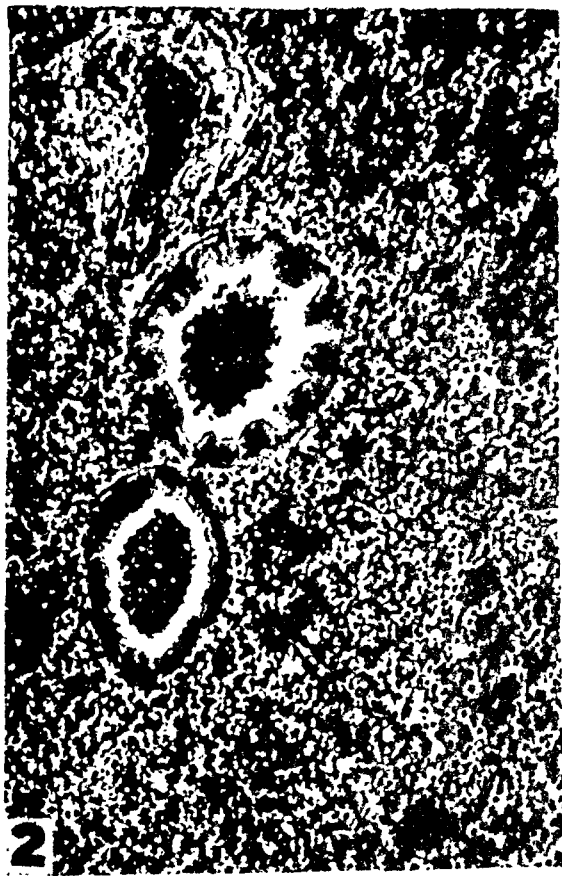
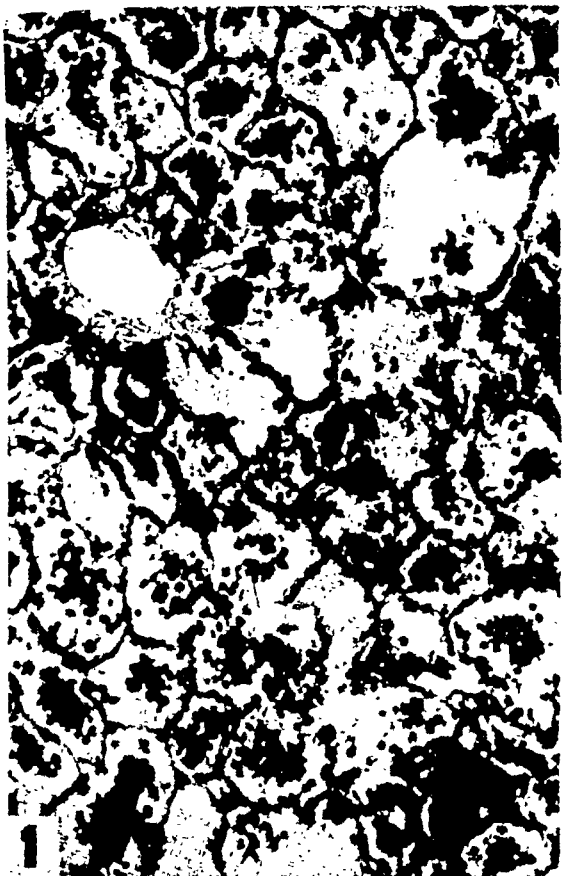
The virus was highly infectious for mice following intracerebral inoculation, but only slightly so following subcutaneous inoculation. Rats, guinea pigs, and rabbits were also susceptible to infection with the virus. In these three species, an encephalitis resulted from intracerebral inoculation; rabbits, however, appeared more susceptible by this route than did guinea pigs and rats.

Following corneal inoculation in the guinea pig, an opacity developed which regressed. In the rabbit, however, the opacity may progress to ulceration of the cornea; and in three out of four animals showing corneal lesions a paralysis of the extremities developed. Intracutaneous injection of three guinea pigs was followed in each instance by the appearance of small nodules in the skin at the site of inoculation. No lesions developed when rabbits were injected intradermally.

Discrete, opaque foci appeared on the surface of the chorio-allantoic membrane of chicken embryos when they were inoculated with the virus.

Histological study of sections from the pons, medulla, and cerebellum of the brain of the child disclosed a meningo-encephalitis, the most conspicuous features of which were areas of necrosis, perivascular cellular infiltration, focal and diffuse inflammatory reaction, and nerve cell degeneration with intranuclear inclusions similar to those seen in known herpetic lesions.

Similar intranuclear inclusions were present in the brains of infected rabbits and rats. None was observed in the sections of the guinea pig brains studied, and few in those of mice. Inclusions were also present in large numbers in the proliferative lesions of the chick chorio-allantoic membrane. In all of the



thetized with nembutal, its abdomen opened and the pancreas removed. Blocks less than 3 mm. in thickness were taken from the head, body and tail of the pancreas and fixed immediately in Zenker-formaldehyde solution and in Bouin's solution. The remainder of the pancreas was used for insulin assay. The animals were then killed and autopsies were usually performed within an hour after death, but this was not always possible. Tissue for sections was obtained from the pituitary, thyroid, parathyroid and adrenal glands, and from the liver, kidney and spleen in each case. These blocks were fixed in Zenker-formaldehyde solution. Additional blocks from the liver and kidney of many cases were fixed in formaldehyde for fat stains and in absolute alcohol for glycogen preparations.

Paraffin sections stained with haematoxylin and eosin were prepared from all the organs mentioned. The blocks of pancreatic tissue fixed in Zenker-formaldehyde solution were sectioned in paraffin at 3 μ . Several sets of sections from the three parts of each pancreas were prepared. One set was stained with haematoxylin and eosin, a second by the neutral ethyl violet-Biebrich scarlet method described by Bowie,¹⁰ and a third by the Mallory-azan technique. Other granule stains were occasionally used. Frozen sections of liver were stained with Scharlach R and paraffin sections of the alcohol-fixed liver and kidney were prepared by both iodine and Best's carmine method for the demonstration of glycogen.

Microscopic Findings in Temporary Diabetes

Islets of Langerhans. Islets in the normal pancreas of the dog stained by the Bowie technique after fixation in Zenker-formaldehyde solution did not, in our experience, provide as clear-cut differentiation of alpha and beta cells as did many of our sections stained by the Mallory-azan technique. In the Bowie preparations both alpha and beta granules were blue. The granules of the alpha cells were somewhat larger than those of the beta cells and they were a deeper blue, or even purple. Alpha granules were more tightly packed than beta granules (Fig. 1). Intermingled with the blue granules of the beta cells was a granular, pink to red staining material thought to be cytoplasm rather than specific granules. Beta cells were much more numerous than

PLATE 123

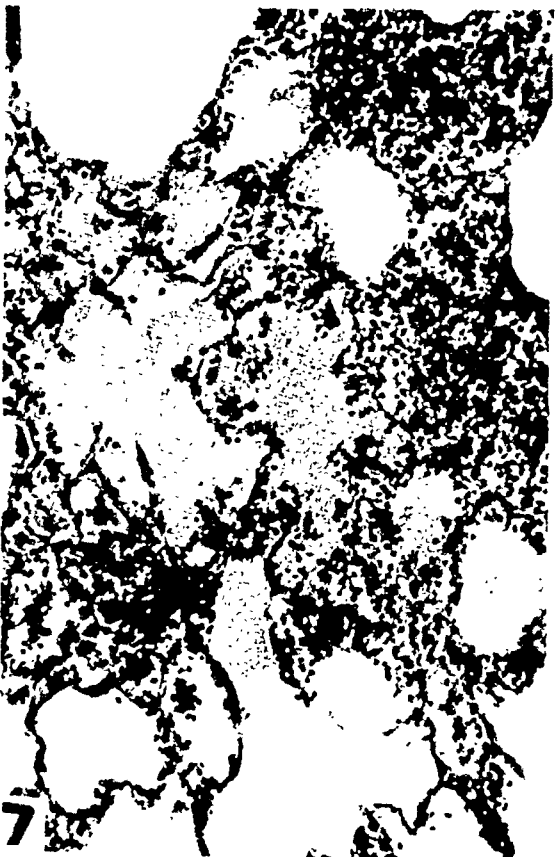
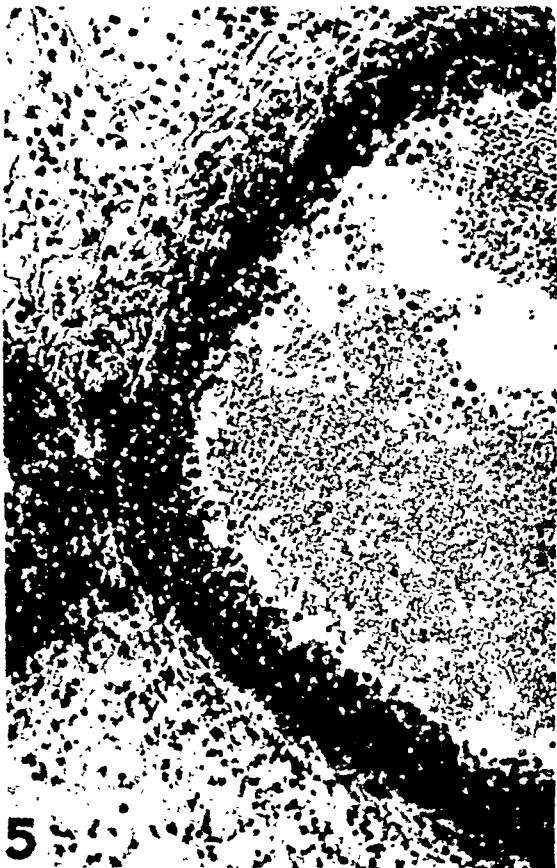
- FIG. 5. From the lung of a rabbit actively sensitized against dried egg white scales 24 hours after intranasal instillation of a solution of dried egg white. Acute phlebitis and acute lymphangitis are seen. $\times 160$.
- FIG. 6. Photomicrograph from the lung of the same rabbit used for Figure 1, demonstrating an early mural thrombus in a pulmonary vein. $\times 275$.
- FIG. 7. Photomicrograph of the lung of a rabbit passively sensitized to crystalline egg albumin 24 hours after intranasal instillation of albumin precipitate. Acute pulmonary edema and acute alveolitis are seen. $\times 120$.
- FIG. 8. Photomicrograph of the lung of the same rabbit shown in Figure 7 to illustrate acute edema and acute hemorrhage into the alveolar and perivascular lymphatic spaces. $\times 130$.

alpha cells, some islets being found which, in a single section, revealed no alpha cells; but usually at least a few alpha cells were evident in any single section of an islet. In our experience it was not always possible to decide whether a cell was an alpha or a beta cell. Most cells were clear-cut, but it was difficult to avoid the impression that there were gradations between the two types. We do not infer here that these elements represent one type changing into the other. Throughout this work we were impressed by the fact that the extent of the blue granulation of the beta cells, as estimated by a study of sections stained by the Bowie technique, bore a close relationship to the insulin content of the pancreas in the different animals. (The insulin content was determined and reported by Best, Campbell and Haist,⁹ 1939.) The Mallory-azan technique, although excellent for differentiating alpha and beta cells, did not give a very satisfactory indication of the extent of beta cell granulation.

Following injections of anterior pituitary extract the first change observed in the islets was a depletion of beta cell granules. Some depletion was observed after 2 daily injections, greater depletion after 4 injections, and after 7 injections it was extremely difficult to find more than traces of beta cell granules in the sections stained by Bowie's method. The beta cell cytoplasm at this time consisted only of the faint pink to red background described previously (Fig. 2). At this time the alpha granules, except in 2 of the dogs, were still clearly visible in the islets.

The early stages of hydropic degeneration were seen in most of the 10 animals injected for 7 days. In 2 of these the degeneration was well developed, as it was in all but 1 of the 4 animals injected for 11 days. Islands containing hydropic beta cells and unaffected alpha cells were frequently seen in the sections stained with granule stains. No clear-cut hydropic degeneration of alpha cells was observed. In its early stages hydropic degeneration was evidenced by clear vacuoles in the cytoplasm of the beta cells. In more advanced stages the cells became swollen with fluid and the cytoplasm reduced to a lacy network extending from the nucleus to the cell membrane. In advanced stages the nucleus tended to be pyknotic and the cytoplasm to be almost completely replaced with fluid (Fig. 3).

The alpha cells appeared to be unaffected in all animals ex-



cept the 2 in which severe hydropic degeneration of beta cells occurred after 7 daily injections of the extract. In 1 of these there was a complete lack of alpha granules and in the other a moderate degree of alpha cell degranulation. The islets in these animals were surrounded by acinar tissue in which the fixation and staining were excellent, hence the lack of alpha cell granules could not be attributed to faulty fixation or staining. These 2 animals in which alpha cells evidenced degranulation were the only ones in which there was an extensive deposition of fat in the liver.

Mitotic figures appeared in the islets (Figs. 5, 6 and 10) as soon as 4 days after the first injection; and up to 11 days were not uncommon. On occasion two were detected in the same islet (Fig. 5). From the haematoxylin and eosin sections the mitotic figures appeared to be in beta cells.

Acini. Mitotic figures were seen in acini as soon as 3 days after injections were begun. They were numerous at 7 days (Figs. 7, 9 and 10) and were still apparent after 11 daily injections. Two mitotic figures in the same oil-immersion field were very commonly found and it was not unusual to find three (Fig. 9).

Ducts. No alterations were observed in the larger ducts. Mitotic figures were found in the smaller ducts (Fig. 8). The small ducts in some of the 7-day animals demonstrated clear vacuoles in the cytoplasm of their cells. This condition was more pronounced in the 7-day animals (Fig. 4).

Undifferentiated Duct Epithelium. Bensley¹¹ described cords and tubules of undifferentiated epithelium which connect with both islets and ducts in the pancreas. On many occasions, cells were seen between acini which we interpreted as belonging to this system. These cells did not contain the specific granules of the islet cells, but single alpha and beta cells were frequently seen in identical situations, suggesting that they had developed from the undifferentiated cells. Without employing quantitative methods it was impossible to establish whether the injections of the extract led to an increase in the number of beta or alpha cells in these locations, or whether the number of undifferentiated cells was greatly increased. Mitotic figures were observed in the latter (Fig. 11), as well as vacuolation (Fig. 4) similar to that seen in the ducts.

Thyroid Gland. Up to 7 days after the beginning of daily injections the follicular cells gradually increased in size and became columnar in shape (Fig. 13). In many of them a vacuolated, light-staining area appeared between the nucleus and the inner cell border. Mitotic figures were common from the third to the seventh day (Fig. 14). Infolding of the walls of the follicles developed in some of the animals between the fourth and seventh days. Up to the seventh day the amount of colloid was gradually reduced and a well marked zone of vacuoles became apparent between the remaining colloid and the inner border of the follicular cells. The colloid stained much less intensely with eosin. After 11 daily injections the histological changes in the thyroid were not so great as in some of the 7-day animals.

Parathyroid Glands. In the parathyroid glands the chief alteration was the proliferation of cells. In the interval between the third and seventh daily injections, mitotic figures were readily observed (Fig. 16). As many as three were found in a single oil-immersion field. Two mitotic figures in the same oil-immersion field were found in many sections. The cytoplasmic changes were not marked. As may be seen in Figure 16, the nuclei in some areas were separated somewhat further from one another by a lighter staining cytoplasm than is normally the case.

Adrenal Cortex. Even though allowing for the fact that it is difficult to ensure that sections of different adrenals are cut at comparable sites and in the same plane, there was considerable variation in the microscopic appearance of the adrenals in animals similarly treated. On the whole, the most marked alteration was in the zona glomerulosa. In the dog this zone is normally of considerable depth and is composed of cells which have paler cytoplasm than those of the adjoining zona fasciculata (Fig. 17). An appreciable number of mitotic figures were found in the zona glomerulosa after 2 daily injections of the extract. In other animals which received up to 7 injections the number of mitotic figures varied considerably but on the whole they were numerous between 4 and 7 days (Fig. 19). It was not unusual to see two in a single oil-immersion field. The cytoplasm of the cells of the zona glomerulosa became increasingly vacuolated up to 7 daily injections and the cells were larger. The depth of the zone was increased (Fig. 18).

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The zona fasciculata was affected to some degree by the extract. Occasional mitotic figures were observed in this region.

Liver. After 1 and 2 injections of the extract, the parenchymal cells were evenly vacuolated to a moderate degree. The carmine and iodine preparations indicated that vacuolation was due to glycogen. After 3 injections of the extract, the parenchymal cells close to the central veins became extensively vacuolated (Fig. 20). After 7 daily injections, areas of the parenchyma were seen in which the constituent cells were greatly swollen with fluid (Figs. 21 and 22). Little cytoplasm was apparent in these cells. Many of them had pyknotic nuclei which were located at the side of the swollen cell. In some cases cell boundaries were broken. In preparations stained by Best's carmine and iodine methods, glycogen was shown to be present in the fluid content of the cells and a few intercellular pools of fluid containing glycogen were scattered about in these areas of swollen cells. In some livers fluid which was stained by the glycogen stains was present in distended portal lymphatics. The sinusoids were apparently obliterated in the areas where the liver cells were swollen. The histological picture in these areas of swollen liver cells presented a striking resemblance to that of the liver in von Gierke's disease observed by Boyd.¹²

After 11 daily injections, the livers of the animals we examined did not present such extensive vacuolation as those of the 7-day animals. In one 11-day animal there were a great many mitotic figures present in the liver.

In two 5-day animals the parenchymal cells of the liver were loaded with fat droplets (Fig. 15). The fat was widely distributed throughout the lobules and each cell contained many droplets. These two livers contained little glycogen. Apart from these 2 animals the fat stains revealed little fat in the livers of the injected animals. The 2 dogs which showed extensive deposition of liver fat were the only ones in which the alpha cells of the islets had been noticeably affected.

Kidney. Several kidney sections were stained for glycogen but only a little was seen in one 7-day animal. Mitotic figures were observed in the loops of Henle in 1 of the animals injected for 11 days.



FRANK BURR MALLORY
(1862-1941)

RECOVERY FROM TEMPORARY DIABETES

Material and Methods

This group consisted of 6 animals which were injected once daily for 7 days and then allowed to recover for 2, 3, 4, 5, 8 and 27 days respectively before they were sacrificed.

The methods employed in this experiment were similar to those described under the heading "Temporary Diabetes."

Microscopic Findings

In the dog sacrificed 2 days after receiving the last of 7 daily injections, the histological picture in various organs differed little from that observed 1 day after 7 daily injections (Fig. 23).

Three days after the last of 7 daily injections, preparations of the pancreas stained by the Bowie method for the first time in this series revealed some well granulated beta cells and some which contained varying degrees of granulation up to normal (Fig. 24). The latter cells gave the impression that the beta granules were somewhat larger and more intensely stained than usual. Some areas in islets at this time demonstrated no beta granulation. The alpha cells were numerous and well granulated. In preparations stained by haematoxylin and eosin, mitotic figures were still seen in the acinar cells of the pancreas but, for the first time in this series, no hydropic degeneration of islet or duct cells was observed. No mitotic figures were observed in the adrenal cortex. The liver cells had somewhat more than a normal content of glycogen and presented a normal appearance. The colloid in the thyroid follicles was more deeply stained than hitherto. The follicles were not completely filled, however, and the epithelium was columnar. One mitotic figure was seen in the section of the parathyroid gland.

Four days after the last of 7 daily injections of the extract, pancreatic tissue prepared by the Bowie method revealed a normal extent of granulation in the beta cells (Fig. 25). The granules, however, seemed to be somewhat larger and darker than usual. In the haematoxylin and eosin preparations, occasional mitotic figures were found in the acinar cells of the pancreas. The thyroid epithelium was still high but the colloid was deep

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FRANK BURR MALLORY

Frank Burr Mallory died on September 27, 1941 at the age of 78. He was a past president of the American Association of Pathologists and Bacteriologists and served as its treasurer from 1911 to 1940. In 1923 he became editor-in-chief of the JOURNAL OF MEDICAL RESEARCH. When that journal became the AMERICAN JOURNAL OF PATHOLOGY in 1925, he served in a similar capacity until 1940. It was under his direction that the AMERICAN JOURNAL OF PATHOLOGY attained the high position it holds among scientific periodicals.

Dr. Mallory graduated from Harvard College in 1886 and received the degrees of A.M. and M.D. from Harvard Medical School in 1890. He became associated with the Boston City Hospital in 1891 and continued this association up to the time of his death. He was made pathologist to this hospital in 1908 and served as such until his retirement in 1932 when he became consulting pathologist. The present pathological laboratory was named, in his honor, the "Mallory Institute of Pathology." He joined the teaching staff of the Harvard Medical School in 1890, was made assistant professor of pathology in 1896, and associate professor in 1901. In 1928 he was appointed professor of pathology, becoming emeritus professor in 1932.

Dr. Mallory was a member of numerous scientific societies in the United States and also was a corresponding member of the Royal Medical Society of Budapest. He received the honorary degree of Sc.D. from Tufts College in 1928 and a similar degree from Boston University in 1932. In 1935 he was awarded the Kober medal by the Association of American Physicians for outstanding service in pathology. He was the third individual to receive the gold-headed cane presented by Dr. Harold C. Ernst

pink. The adrenal gave no histological indication of heightened activity.

Five days after the last of 7 daily injections, the thyroid epithelium was still higher than normal and some mitotic figures were found in the adrenal cortex. Other tissues evidenced no abnormalities.

Eight days after the seventh daily injection, the thyroid gland demonstrated follicles lined by cuboidal epithelium and well filled with deep pink colloid. The liver contained a large amount of glycogen.

Twenty-seven days after the seventh daily injection, the various endocrine glands examined presented pictures within the range of normality. The islets of Langerhans, however, exhibited slightly more collagen in association with their vessels than usual.

PERMANENT DIABETES

Material and Methods

Tissue was obtained at operation from the head, body and tail of the pancreases of 3 dogs. It was fixed in Zenker-formaldehyde and Bouin's solutions. Sections were prepared by the same technique as described under the heading "Temporary Diabetes."

One dog received daily intraperitoneal injections of an anterior pituitary extract for 21 days and the other 2, for 30 days. Administration of insulin was started 30 to 59 days after the last injection of the extract. Tissue was obtained at periods ranging from 78 to 198 days after the last injection of anterior pituitary extract. Best, Campbell and Haist⁹ have reported that these 3 dogs were permanently diabetic and that their pancreases contained little insulin.

Microscopic Findings

The three pancreases exhibited one feature in common, a lack of granular beta cells. Aside from this fact the three pancreases differed considerably in their microscopic appearance.

Sections of the first pancreas, stained by haematoxylin and eosin, revealed no typical islets. Only small groups of fibroblasts,

to the American Association of Pathologists and Bacteriologists to be awarded for special merit.

Dr. Mallory's publications were numerous and covered a wide range of subjects. His first paper appeared in 1892 and his last in 1939. Among his outstanding contributions were studies on the classification of tumors, cirrhosis of the liver, various technical methods and the pathology of infectious diseases. In 1897 he published with J. Homer Wright the first edition of "Pathological Technique" which went through eight editions. A final revision appeared in 1938. "The Principles of Pathologic Histology" was published in 1914.

Aside from his contributions to pathology, Dr. Mallory made a unique contribution to the field of medicine as a whole in the training of young men. The graduates from his laboratory during the period from 1895 to 1932 numbered approximately 125. This does not include graduate students, National Research Fellows or Rockefeller Fellows. He took a keen personal interest in the development, education and welfare of the members of his staff. Even the youngest member had close contact with him and received the benefit of his advice and criticism on every aspect of his work. By such an association, each individual had impressed on him the value of scientific accuracy and a horror of careless technic. The roster of the graduates of the laboratory includes the names of numerous outstanding men, not only in the field of pathology, but also in the clinical branches of medicine. When, in 1933, a special number of the AMERICAN JOURNAL OF PATHOLOGY was issued by the Assistant Editorial Staff in honor of Dr. Mallory, the authors of the included papers were all graduates of his laboratory who had remained in Pathology.

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which bore only a superficial appearance to islets, were found (Fig. 28). The Bowie preparations of this pancreas revealed only occasional islet cells scattered through the acinar tissue. The granules in these cells resembled those of alpha cells. The Mallory-azan preparations showed increased connective tissue, which had a diffuse distribution. The acinar tissue was somewhat atrophic in appearance and the smaller ducts were not as prominent as usual.

The second pancreas revealed islets which in the haematoxylin and eosin preparations appeared almost normal. They were not present, however, in nearly as great numbers as in a normal pancreas. Granule stains revealed that these islets consisted chiefly of alpha cells (Figs. 26 and 27). There was a marked scarcity of granular beta cells in this pancreas. From Mallory-azan preparations it was found that a slight diffuse fibrosis was present and that the islets had more collagen about their blood vessels than was usual. The acinar tissue appeared to be normal.

The third pancreas was removed from a permanently diabetic animal which had been hypophysectomized a few weeks before autopsy. Histological examination of the brain revealed, however, that the hypophysectomy was not complete, as a small area of anterior lobe tissue was found in the serial sections. Sections of the pancreas of this animal, stained by haematoxylin and eosin, showed a slight to moderate diffuse fibrosis. Islets were seen but they were not as numerous as in a normal pancreas. Some hydropic cells were present in the islets. Granule stains revealed few granular beta cells in the islets and many alpha cells. The most striking finding in this pancreas was the extensive hydropic change in the cells of the smaller ducts. This change was not present in the pancreases of the other two permanently diabetic animals.

DISCUSSION

The Histological Basis for "Permanent" Diabetes

Richardson and Young⁷ were the first to make histological studies on animals made permanently diabetic by anterior pituitary injections. In 1938 they reported their findings in the pancreatic tissue of 2 permanently diabetic dogs. Next, the pancreas of a permanently diabetic dog prepared by Campbell and Best¹³

HISTOLOGICAL STUDY OF TROPHIC EFFECTS OF DIABETOGENIC ANTERIOR PITUITARY EXTRACTS AND THEIR RELATION TO THE PATHOGENESIS OF DIABETES *

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Although the high incidence of diabetes in humans with tumours of the anterior lobe long gave intimation that this lobe of the pituitary gland might possess a diabetogenic function, its rôle in this respect was not definitely established until Houssay and Biasotti¹ in 1930 showed that diabetes, produced in animals by removing the pancreas, could be ameliorated in turn by removing the pituitary gland. Subsequently much information on the relationship between the anterior lobe of the pituitary and diabetes has been obtained by injecting extracts of the anterior lobe into animals. Soon after the experiments of Houssay and Biasotti, three groups of workers produced diabetes in animals by injecting extracts: Evans, Meyer, Simpson and Reichert² (1932), Baumann and Marine³ (1932) and Houssay, Biasotti and Rietti⁴ (1932). But, even before Houssay and Biasotti, Johns, O'Mulvenny, Potts and Laughton⁵ (1927) had recorded that they had produced hyperglycaemia, glycosuria and polyuria in dogs by the injection of an anterior lobe extract.

The next step in the development of knowledge regarding the relationship of the anterior pituitary to diabetes was of still greater significance to the pathologist. It concerned the discovery that anterior pituitary extracts possess, in addition to their ability to produce a diabetic state during the course of injections, ability to bring about a condition of permanent diabetes associated with islet lesions—a condition which persists indefinitely after the injections are discontinued. The work which led to the establishment of this fact will be briefly reviewed.

In their experiments in 1932, Evans and co-workers² observed that two dogs which became diabetic during the course of injections remained diabetic after the injections were discontinued. One recovered after 2 months; the other dog was still excreting

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(1938) was studied by both Robinson and Warren, their findings being briefly reported in the paper by Campbell and Best. Warren¹⁴ has also independently discussed this tissue and the tissue from 2 of Young's dogs. Dohan and Lukens¹⁵ have published a brief report on the histological findings in the pancreases of 2 permanently diabetic dogs and Richardson¹⁶ has recently given data on 3 more.

The various pancreases examined by these workers and the three in our own series have one point in common, namely, a scarcity of islets. Granule stains used by Richardson¹⁶ and also in the present study indicate, furthermore, that the scarcity of granular beta cells is even more pronounced than the scarcity of islets.

In other respects the various pancreases which have been studied differ from one another. For instance, Richardson¹⁶ appears to have found a greater incidence of hyalinization of islets than has been observed in the others, as well as more examples of duct vacuolation. We are inclined to agree with Richardson's view that vacuolation of the smaller ducts is the most characteristic finding in the pancreas after anterior pituitary extracts are injected, but we base our conclusions on our findings in temporary diabetes. After the injections had ceased we found duct vacuolation to be less common. It was present in only one of the three pancreases examined by us. Hydropic degeneration of islets was observed in only a few of the pancreases studied by Richardson. It was evident in the pancreas of the first dog made permanently diabetic by Campbell and Best¹³ and it appeared in only 1 of the 3 dogs we studied. Like the vacuolation of the smaller ducts, however, we found that hydropic degeneration was characteristic of a certain phase of temporary diabetes, rather than of permanent diabetes. We suspect that its absence in permanent diabetes may be due to an absence of beta cells capable of becoming hydropic. The amount of diffuse fibrosis in the various pancreases studied seemed to vary considerably. We gained the impression that in association with it there was atrophy of the smaller ducts.

To sum up, the pancreases obtained from dogs made permanently diabetic with anterior pituitary extracts revealed a scarcity of islets. With granule stains it has been evident that the lack

sugar 4 months after the last injection, when their report was written. The importance of these findings was not generally appreciated until Young⁶ in 1937 showed conclusively that a sufficiently severe course of anterior pituitary injections would make an animal diabetic, not only during the injections but indefinitely. Richardson and Young⁷ found that this permanently diabetic state was associated with islet lesions.

From the foregoing it is apparent why two terms regarding the conditions caused by anterior pituitary extracts; namely, *temporary* (or transient) diabetes and *permanent* diabetes, have come into use. *Temporary* diabetes refers to the state produced by a less severe course of injections after which recovery is effected when the injections are stopped. *Permanent* diabetes refers to the condition which follows a more severe course of injections and which persists after the injections are discontinued.

Through the courtesy of C. H. Best, in whose department studies on aspects of anterior-pituitary-induced diabetes, other than the histopathology, were being made on a large series of animals, we were able to obtain animals in almost every stage of the condition for pathological study. These dogs were of three series representing (1) almost every stage of temporary diabetes, (2) several stages of recovery from temporary diabetes and (3) permanently diabetic animals.

TEMPORARY DIABETES*

Material and Methods

The animals used in these experiments were dogs of different ages, weights and breeds. The details regarding their treatment, the type of anterior pituitary extract used and the dosage, are given in a separate paper by Best, Campbell and Haist⁹ (1939), in which also the results of insulin assays on the pancreases of many of these dogs can be found.

Anterior pituitary extract was given by subcutaneous injection. The first dog was killed 8 hours, and a second 24 hours, after a single injection. Another dog received 3 daily injections, another received 4, 10 received 7 and 4 received 11 daily injections. Twenty-four hours after the last injection, each dog was anaes-

* The material used for a preliminary report⁸ is included in this study.

of granular beta cells was almost complete. Hence it seems reasonable, for the time being, to adopt the position that permanent diabetes is due to a lack of insulin production. Why anterior pituitary extracts should bring about this state of affairs will be discussed next, in the light of our findings in temporary diabetes.

The Cause of the Diabetic State in Temporary Diabetes

Unlike permanent diabetes, temporary diabetes cannot be explained by the single factor of an absolute reduction in insulin production.

We found that daily injections of anterior pituitary extract produced a progressive degranulation of the beta cells and that this change was closely followed by hydropic degeneration. Our findings in pituitary-injected animals are identical with those observed years ago by Homans¹⁷ and Allen¹⁸ in pancreatic remnants, when enough of the pancreas had been removed to cause diabetes. In his long series of experiments, Allen offered convincing evidence that hydropic degeneration of the beta cells in the partially depancreatized animal was caused by their being called on to perform work beyond their capacity. Since the changes we observed in animals injected with anterior pituitary extracts so closely resembled those he found in the partially depancreatized animal and as they developed in the same sequence, we conclude that they result from the same cause, namely, excessive stimulation of activity. Hence we suggest that the reason anterior pituitary extracts can cause islet damage is because of their ability somehow to stimulate beta cells to overwork.

To attribute the beta cell lesions that we observed to overwork has interesting implications. First, from Allen's¹⁸ work in partially depancreatized animals, it seems clear that hydropic degeneration does not develop until beta cells are called on to perform many times their usual amount of work. For example, removing two-thirds of the pancreas does not result in hydropic degeneration in the remaining fragment; for this to occur four-fifths to nine-tenths of the pancreas must be removed. It would appear from this that beta cells must be called on to perform five to ten times their normal work before they show hydropic degeneration. Hence in our dogs in which, in contrast to the

experimental animals the cerebral lesions were essentially the same, although varying in intensity.

Cross neutralization was complete in tests performed with known herpes virus and antiserums. These results, together with those of the histological study and with the susceptibility of experimental animals, lead to the conclusion that the agent isolated is herpes simplex virus.

The work of Dodd, Johnston, and Buddingh,¹⁶ and of Burnet and Williams,¹⁷ has definitely shown the importance of aphthous stomatitis in children as a primary herpetic infection. It seems, therefore, that infants and children are highly susceptible to infection with herpes simplex virus, and one might anticipate that involvement of the central nervous system would be most likely to occur at this age. However, a review of the literature shows that this is the first report of a case of encephalitis from which herpes simplex virus was isolated in which the etiological significance of the virus has been established by the demonstration of typical herpetic inclusions in the human brain tissue.

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DESCRIPTION OF PLATE

PLATE 13

FIG. 1. Focus of proliferated glia in pons. This and all other figures were made from sections of human brain. $\times 100$.

FIG. 2. Area of necrosis and inflammation in pons. Perivascular infiltration of lymphocytes. $\times 100$.

FIGS. 3 and 4. Nerve cells in pons showing large intranuclear inclusions separated from nuclear membranes by clear zones. $\times 720$.

FIG. 5. Nerve cell in pons showing large granular inclusion filling the nucleus. $\times 720$.

FIG. 6. Nerve cell in cerebellum showing a large granular inclusion filling the nucleus. Dots of nuclear chromatin on the nuclear membrane. $\times 720$.

partially depancreatized animal, the whole pancreas was present, it might be concluded that before widespread hydropic degeneration appeared the total insulin liberation of each pancreas was many times the normal amount. It is necessary, therefore, to explain why this greatly increased insulin output was not associated with a great reduction in the blood-sugar level but instead with a slight elevation in the blood-sugar level during the first few days of injections (for blood sugar curve see Best, Campbell and Haist⁹).

To answer this question we refer again to the work of Houssay and his associates.^{1,4} When they discovered that diabetes resulting from pancreatectomy was ameliorated by hypophysectomy, they laid the basis for the conception of diabetes as a disease due to a disturbed relationship between anterior pituitary and beta cell activities rather than as one which results when insulin production falls below a certain absolute minimum. To carry this thought a little further, if the absence of diabetes depends upon the constancy of the ratio,

$$\frac{\text{diabetogenic activities of (or controlled by) anterior pituitary}}{\text{antidiabetogenic activity of beta cells (insulin secretion)}}$$
then diabetes could be seen to be present or absent over a wide range of absolute values for the two factors in the ratio. In the case of the animals in the early stages of temporary diabetes, both factors were generally increased. But because of the great increase in the numerator, the increased denominator (insulin secretion) was not effective in bringing about hypoglycaemia. Indeed, at best it was only able approximately to balance the increased numerator for a few days, after which it became unable to compensate further for the increased numerator and the diabetes became greatly accentuated.

The Cause of the Excessive Stimulation of Beta Cells Observed After the Injection of Anterior Pituitary Extract

There are two ways in which anterior pituitary extracts could stimulate beta cells: *indirectly* and *directly*. The *indirect* method would depend on effects of the extract exerted elsewhere than the pancreas, which increase the need of the organism for insulin. The *direct* method would depend on the existence in the extract of a principle which directly stimulates beta cell secretion. From

In all animals, the extent of beta cell granulation as depicted by the Bowie technique, closely paralleled the insulin concentration in the pancreas as reported by Best, Campbell and Haist.

CONCLUSIONS

1. Anterior pituitary extracts, injected in daily doses into dogs, cause progressively, degranulation, hydropic degeneration and death of the beta cells by stimulating these cells to excessive function.

2. Anterior pituitary extracts may act in two general ways to cause beta cells to overwork:

(a) By acting on tissues and organs other than the pancreas so as to increase the body's need for insulin. Such actions include increasing the amount of carbohydrate to be metabolized, and also making insulin relatively ineffective.

(b) By exerting a "trophic" effect on the pancreas. This is indicated by proliferative changes (mitotic figures in various epithelial elements, including the beta cells). It may also be a factor in permitting the beta cells to secrete at a destructively high rate, since trophic principles in general seem to affect secretion as well as growth.

3. Diabetes is seen to be due, not to a decrease in a fixed production of insulin, but rather to an increase in the $\frac{\text{insulin needs}}{\text{insulin production}}$ ratio.

4. The early stages of diabetes produced by anterior pituitary extracts can be explained primarily by increased needs for insulin.

5. The later stages of diabetes produced by extracts (permanent diabetes) can be explained primarily by diminished production of insulin.

6. It is evident that, experimentally at least, there are two general types of diabetes: one due primarily to increased insulin needs, the other primarily to decreased insulin production. It is also evident that diabetes due primarily to increased needs may lead to exhaustion of beta cells and hence decreased production.

7. Beta cells, stimulated to excessive activity for only a few days, will recover if the injections of extract are discontinued,

our studies it seems reasonable to conclude that beta cells were stimulated by both methods.

1. *Indirect Stimulation.* One anterior pituitary effect which would indirectly stimulate beta cells by creating increased need for insulin is the effect on the thyroid. The histological picture in this gland indicated that under the influence of the injected anterior pituitary extract it became extremely active. The state of hyperthyroidism caused by the extract would have a definite effect on increasing the need for insulin.

A second effect of the extract which would indirectly stimulate beta cell activity by increasing insulin need was observed in the adrenal cortex. Here, as in the thyroid, the histological picture indicated great functional activity (Figs. 18 and 19). Long, Lukens and their associates¹⁰ have offered much evidence which indicates that the cortical hormone stimulates the formation of sugar from protein. Such an effect would increase the body's need for insulin.

A third effect of the extract was observed in the liver. Housay²⁰ stated that the liver is the only organ in the body, the removal of which will prevent the diabetogenic effects of anterior pituitary extracts. In this work we have described a characteristic liver lesion which developed when the extract was given in daily injections. This lesion consisted essentially of a hydropic state of the liver cells, associated with loss of their granular cytoplasm. The fluid in these distended cells contained glycogen. Warren¹⁴ stated that some of the larger livers he had studied in human diabetes suggested hydrops of the liver cells. The histological picture of the liver cells in the lesions we observed closely resemble those in the liver of a case of von Gierke's disease illustrated by Boyd.¹²

As far as could be judged by our glycogen stains, the glycogen content of the liver, which was excellent at the beginning of the experiment, altered only to become slightly increased as the injections continued. As the liver was on a positive glycogen balance, some of the extra glucose in the blood must have been derived from other than carbohydrate sources, probably from protein. The manufacture of sugar from materials other than carbohydrate takes place in the liver and it is possible that the peculiar hydropic state of many of the liver cells is related to

but will not recover when stimulated to excessive activity for a sufficiently long time.

8. That the cells of the smaller ducts and cords of undifferentiated epithelial cells in the pancreas become hydropic during the course of injections is considered to be an important factor in a vicious circle which operates to interfere with an adequate regeneration of beta cells once diabetes is established.

NOTE: We wish to express our appreciation to C. H. Best for suggesting that this study be made and for his courtesy in providing the animals used.

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their activity in making sugar from noncarbohydrate materials. This activity of the liver cells may not be due solely to the direct action of an anterior pituitary principle.*

One result of the described effects of the anterior pituitary extract would be to increase the sugar which the body must metabolize (sugar from protein and possibly from fat). Hence if insulin is needed for the metabolism of carbohydrate, this effect would increase the body's insulin requirements.

However, it appears probable that anterior pituitary extract increases insulin need in an additional way concerned with rendering insulin ineffective. Many workers have shown that shortly after receiving anterior pituitary injections animals exhibit a reduced response to injected insulin despite the fact that the blood sugar is not yet elevated (Houssay and Potick,²¹ Cope and Marks²²). If insulin secretion is controlled by the blood-sugar level, the insulin resistance observed by these workers would not place any increased strain on the beta cells since the blood-sugar level was not raised in their experiments. It would place a strain on the beta cells, however, if insulin secretion is controlled by the level of insulin in the blood. In support of this method of control, we found that beta cell secretion, as indicated by the state of granulation, was greatly stimulated in the early phases of temporary diabetes at a time when the fasting blood-sugar levels were not high. Furthermore, Allen²³ showed that partially depancreatized phloridzinized dogs developed the typical islet cell lesions as readily as partially depancreatized dogs not given phloridzine, despite the fact that the blood sugar was not elevated. It would appear, therefore, that beta cells can be strained in the absence of a diabetic blood-sugar level. The evidence suggests that insulin secretion may be controlled primarily by the blood-insulin level. This cannot be definitely established until methods of measuring the blood-insulin level are available. If insulin secretion is controlled by the blood-insulin level, it is easy to see that any anterior pituitary effect which acts to metabolize insulin would lower the blood-insulin level and stimulate beta cell secretion.

* The work of Long and his associates¹⁰ suggests that the hormone of the adrenal cortex is involved in this effect also, but Houssay²⁰ and his co-workers offer evidence in some of their experiments that the effect can be produced in the absence of the adrenals.

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2. *Direct Stimulation.* It seems advisable to point out that there is no clear-cut concept of what constitutes a "trophic" effect. The word refers to nutrition and has been applied to growth. However, it is obvious that secretion, as well as growth, is stimulated by many, if not all, of the so-called "trophic" principles present in anterior pituitary extracts. For instance, loss of colloid is one of the characteristic effects of the thyrotropic principle on the thyroid gland (Fig. 13). The two phenomena, of increased secretion and growth, parallel each other in so many endocrine glands stimulated by the injection of "trophic" principles that it seems unwise, until further information is obtained, to visualize these principles as stimulating only growth. As to growth and secretion, it appears more probable that an action primarily affecting secretion would be more likely to induce growth than *vice versa*. Much more information is needed on this important subject, but from our findings in most of the endocrine glands we wish to emphasize the secretory effect of the pituitary principles in addition to their proliferative actions.

In 1933 Anselmino, Herold and Hoffmann,²⁴ described an anterior pituitary extract which on injection into rats led to an increase in islet tissue. (Anterior pituitary extracts do not cause diabetes in rodents unless part of the pancreas is removed. There is a marked difference among species with regard to their response to the same extract.) Some workers have confirmed and others have failed to reproduce their results, but Richardson and Young²⁵ have shown that crude preparations of anterior pituitary extract will lead to an increase in the weight of the pancreas and to an increase in islet tissue. Recently, Marks and Young²⁶ have shown that repeated injections of an anterior pituitary extract will lead to an increase in the insulin content of the pancreas of the rat.

We have pointed out that anterior pituitary extracts induce growth in all parts of the pancreas. (Figs. 5-11 illustrate these growth effects.) But we question whether growth is the only change brought about by the action of the pancreatropic principle in the dog. If it is like other trophic principles which affect endocrine glands, it would stimulate secretion as well. So, although excess secretory function of beta cells in the pituitary-injected animals can be explained, in part at least, by an attempt to pro-

effects of continuous intravenous injection of dextrose in increasing amounts on the blood sugar level, pancreatic islands and liver of guinea pigs. *Ibid.*, 1939, 75, 91-105.

31. Sergeyeva, M. A. Microscopic changes in the islands of Langerhans produced by sympathetic and parasympathetic stimulation in the cat. *Anat. Rec.*, 1940, 77, 297-317.
32. Ham, A. W. Cartilage and Bone. In: Cowdry, E. V. *Special Cytology*. Paul B. Hoeber, Inc., New York, 1932, ed. 2, 981-1051.

DESCRIPTION OF PLATES

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FIG. 1. Oil-immersion photomicrograph of an islet in a Bowie preparation of pancreas obtained from a dog which received 1 injection of extract 8 hours before it was killed. Two alpha cells can be seen on the left side of the islet, recognizable by their dark granules. The other dark-staining material slightly below the center of the islet is a capillary and its supporting reticulum. The remainder of the cells in the islet are beta cells and their fine granulation is apparent.

FIG. 2. Oil-immersion photomicrograph of an islet in a Bowie preparation of pancreas obtained from a dog which received 7 daily injections of the extract. Acinar cells with zymogen granules mark the left border of the islet. Close to the center some alpha cells, well filled with their coarse, dark granules, are apparent. The remainder of the cells in the islet are beta cells. Their blue granulation has almost completely disappeared; nothing remains but the pale cytoplasmic background. Some of the beta cells are hydropic.

FIG. 3. Islet from a dog which received 11 daily injections of the extract. The preparation is stained with haematoxylin and eosin. Many of the islet cells are hydropic.

FIG. 4. Medium power photomicrograph of an area of pancreas obtained from a dog which received 7 daily injections of the extract. The section is stained with haematoxylin and eosin. Hydropic degeneration is evident in several islets and in smaller ducts and cords of undifferentiated epithelial cells. Continuity of an islet and a small duct is obvious in one instance.

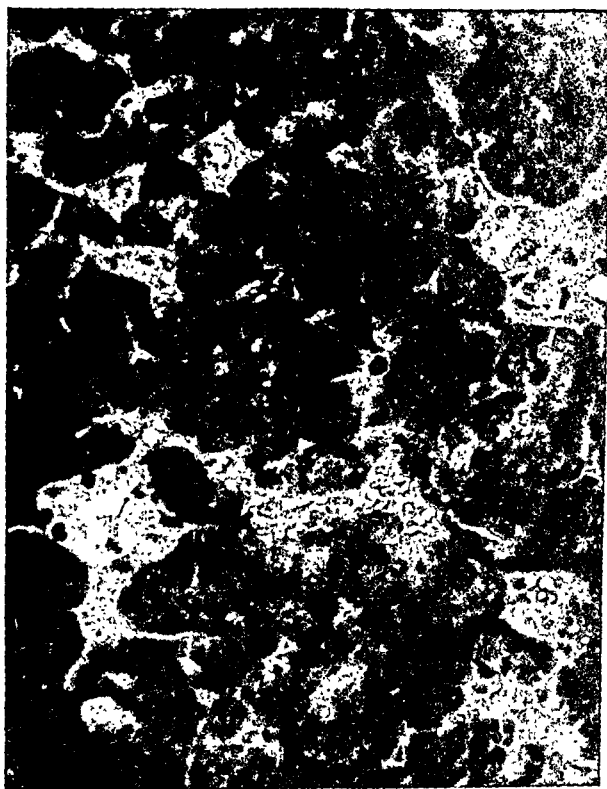
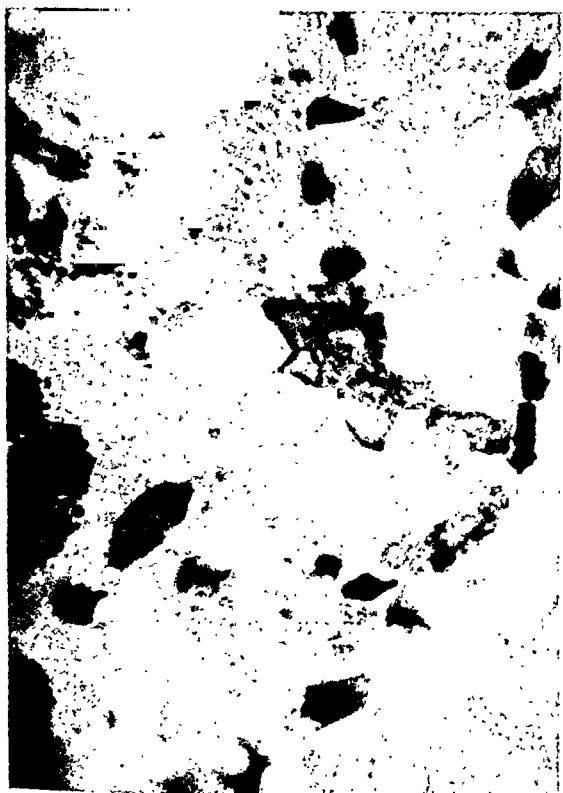
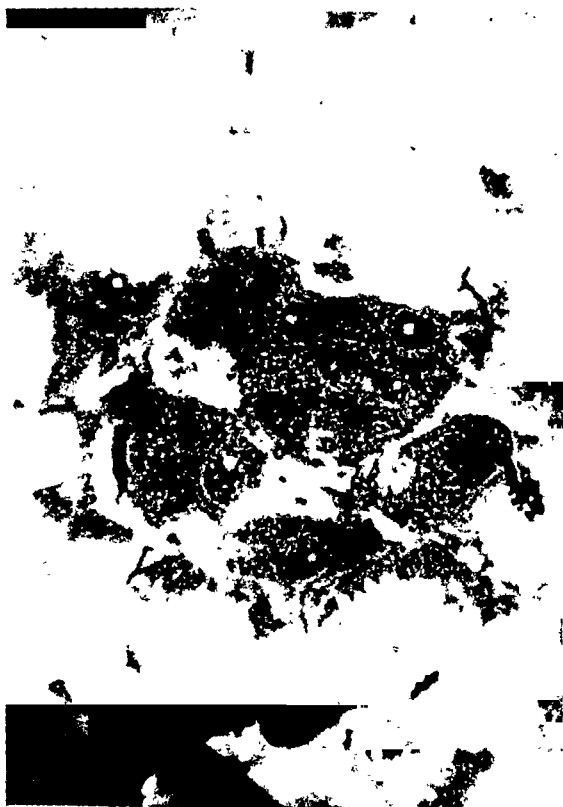
vide for the increased need for insulin, we think that direct stimulation of secretory activity by the pancreatropic principle may be an important factor in causing the exhaustion of beta cells. It was our impression from the various endocrine glands studied, that trophic principles act by removing restraints on endocrine gland-cells which ordinarily hold their secretory and growth activities within certain limits. This is not a permanent effect as we found that trophic stimulation usually became less effective as injections continued. After a certain amount of trophic stimulation, the cells in most glands resumed a more normal appearance. But in the effective phase of trophic stimulation conditions might permit cells to attempt work beyond their physiological limits. In the absence of trophic stimulation it seems doubtful that a few days of ordinary overwork would produce the profound islet lesions observed. Hence it seems possible that trophic stimulation of beta cells may be a factor in causing or permitting the excessive overwork which leads to their exhaustion.

*The Relation of the Injected Extract to the Alpha
Cells and Liver Fat*

The alpha cells of the islets appeared much more immune to the effects of the extract than the beta cells. In only 1 animal was there an almost complete degranulation of alpha cells. This animal showed extensive depositions of fat in the liver. The only other fatty liver in the series was obtained from an animal which showed a moderate degranulation of alpha cells. The coexistence of fatty livers and alpha cell degranulation in these 2 animals may be only a coincidence, but because of the recent interest in the relation of the pancreas and anterior pituitary to fat metabolism it merits mention.

Possible Types of Diabetes and their Relation to Islet Lesions

That pancreatectomy produces diabetes, that islet lesions are found in many human cases of diabetes and that insulin relieves diabetes have encouraged the view that diabetes is a disease due to an absolute reduction in insulin production. But the equally impressive fact that a large percentage of humans with diabetes demonstrate no islet lesions at autopsy has always stood in the



way of the unquestioning adoption of this concept. The relatively recent work in connection with the anterior pituitary and diabetes has led to the development of a somewhat different concept of the cause of the disease and may give some intimation why some cases exhibit islet lesions while others do not.

Sufficient evidence has been presented in this paper to show that diabetes may be due not to a simple reduction in insulin liberation but rather to an increase in the $\frac{\text{insulin needs}}{\text{insulin production}}$ ra-

tio. An increase in the ratio, and hence diabetes, is theoretically possible, because of (1) a decrease in insulin production and liberation below needs, or (2) an increase in needs beyond production and liberation of insulin. Thus it is theoretically possible that diabetes could result primarily from either *pancreatic* or *extrapancreatic* causes. For example, temporary diabetes produced by extracts is, in its early phases, extrapancreatic diabetes because it depends primarily on insulin needs being increased. Permanent diabetes is, however, pancreatic diabetes as there is islet damage and decreased liberation of insulin.

Our work shows how diabetes resulting from increased insulin needs (extrapancreatic diabetes) can terminate as pancreatic diabetes with islet lesions. This is due to beta cells endeavouring to balance the $\frac{\text{insulin needs}}{\text{insulin production}}$ ratio whenever needs are increased. In the animals studied, very large amounts of extract were injected and the needs were enormously increased. Furthermore, there was evidence of considerable pancreatropic effect of the extract, which we think may be an important factor in permitting gross overwork of the beta cells. It is conceivable that conditions might arise in man which would lead to an increase in insulin needs but with which there would be no undue secretion of pancreatropic principle and hence no exhaustive overwork of beta cells.

It is too soon to be certain why some humans with diabetes show no islet lesions. First, pancreatic tissue from more human cases with apparently normal islets needs to be investigated by means of granule stains to make sure these apparently normal islets are not constituted almost entirely of alpha cells or agranular beta cells, as in permanent diabetes in dogs. Second, it is

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FIGURES 5 to 11 inclusive illustrate the pancreatropic effect of the extract.

FIG. 5. Oil-immersion photomicrograph of a section stained by haematoxylin and eosin from a pancreas obtained from a dog which had received 4 daily injections of the extract. Two mitotic figures are present in this islet: one in the midline close to the border of the acinar tissue; the second above it, is close to the apparent centre of the islet.

FIG. 6. Preparation same as for Figure 5 showing mitotic figure in islet.

FIG. 7. Preparation same as for Figure 5 showing two mitotic figures in acinar cells.

FIG. 8. Preparation same as for Figure 5 showing mitotic figure in cell of small duct.

FIG. 9. Preparation same as for Figure 5 showing three mitotic figures in acinar cells in the same oil immersion field.

FIG. 10. Preparation same as for Figure 5 showing one mitotic figure in islet above, and one in acinar cell below.

FIG. 11. Preparation same as for Figure 5 showing two mitotic figures in a cord of undifferentiated epithelium in the centre of the field.

possible that there is a condition, as yet not understood, in which insulin is somehow prevented from exerting its action, without being metabolized, so that it remains in the circulation and inhibits secretion by the beta cells which are therefore undamaged. Third, humans with diabetes who show no islet lesions may possibly be those in whom the condition is primarily due to increased insulin needs but in whom the factors necessary to produce exhaustive overwork of beta cells are lacking (e.g., enough of the pancreatropic factor or a poor beta cell potentiality).

Whether it is possible to distinguish clinically between diabetes due primarily to increased insulin needs and that due primarily to diminished insulin production is a matter outside the scope of this paper. Himsworth²⁷ and Graham²⁸ have recently discussed the problem of two clinical types of human diabetes and the problems arising therefrom.

Beta Cell Regeneration

It is useless to speculate at present whether the islet lesions evident in most cases of diabetes are due to the beta cells attempting to compensate for unusual insulin needs or to their inability to compensate for ordinary needs. In any case, beta cell potentiality must be equivalent to the insulin needs of the individual if diabetes is to be absent. It seems desirable, therefore, to comment briefly on the problem of beta cell regeneration, particularly as we have obtained some evidence to indicate a vicious circle, in that, beyond a certain point, extra work put upon beta cells operates to prevent their new formation.

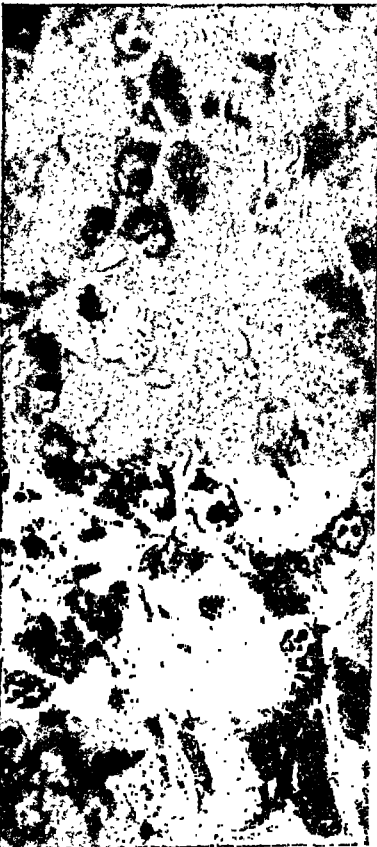
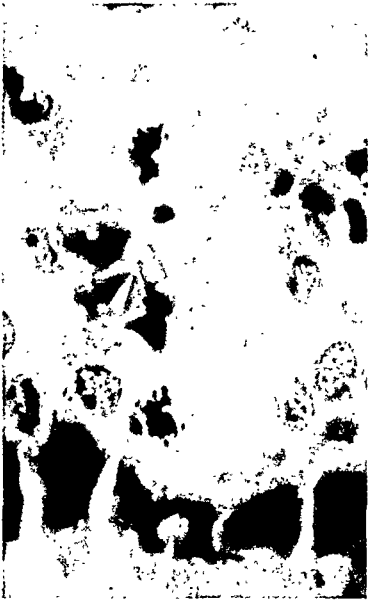
Our results indicate that the islets can recover after a few days of severe diabetes. Degranulated beta cells recovered their granules in 3 days and even moderately hydropic cells recovered, so far as we could tell. But in the dog, if frank diabetes can be prolonged by continuing the injections, the animal becomes permanently diabetic due, we presume, to beta cells being injured beyond the point of recovery. Recovery of function of the pancreas after this depends upon the formation of new beta cells.

When, because of overwork, the beta cells are injured beyond the point of possible recovery, regeneration of new beta cells becomes unusually difficult. The injured beta cells are certainly

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not a good source of new cells. What is perhaps even more important is that stem cells—those of the smaller ducts and cords of undifferentiated epithelial cells—also become hydropic. That the stem cells become vacuolated as well as the functioning differentiated beta cells, may be an important reason why a diabetic condition tends to persist. (Allen¹⁸ observed duct vacuolation after partial pancreatectomy and stressed its importance in inhibiting the formation of new islets. Richardson,¹⁶ as well as ourselves, has observed it after anterior pituitary injections and stresses its importance with regard to islet regeneration).

It is readily apparent that for untreated pancreatic diabetes each new beta cell which the pancreas manages to produce would be overworked and probably destroyed as soon as it develops. It might be thought that this could be prevented by giving insulin, but it is possible that supplying adequate amounts of exogenous insulin may remove, to a great degree, the stimulus for beta cell regeneration. Successful beta cell regeneration seems to require a stimulus for regeneration without excessive strain on any newly formed beta cells. An attempt to obtain these two requirements was recently made by Campbell, Haist, Ham and Best,²⁹ who, in a preliminary report, stated that the administration of insulin with anterior pituitary extract prevented, to a considerable degree, the development of degenerative changes in beta cells; yet the production of new islet cells, as witnessed by mitotic figures, was still observed.

Whether the potentiality for beta cell formation is lost in the pancreas of a dog which has suffered for some time from the permanent type of diabetes, is an important question. If this potentiality is not lost and if experimental means can be found to stimulate the formation of new beta cells without causing their subsequent overwork, there would be hope of its application in the treatment of human diabetes. It is a great advance that animals can be made permanently diabetic, for this provides suitable experimental material for study. But, until it can be shown that anterior pituitary extracts can cause new beta cells to form in a permanently diabetic animal, it would be unwise to regard the pancreatropic effect of anterior pituitary extracts, observed in previously normal animals, as the answer to beta cell regeneration. The smaller ducts in the pancreases of 2 of the

FIG. 12. High power photomicrograph of thyroid gland from a control dog, haematoxylin and eosin stain.

FIG. 13. Medium power photomicrograph of thyroid gland from a dog which received 4 daily injections of the extract. A loss of colloid, heightened epithelium and infolding of follicular walls are apparent. This illustrates the thyrotropic effect of the extract.

FIG. 14. Oil-immersion photomicrograph showing mitotic figure in the same preparation as used for Figure 13.

FIG. 15. Low power photomicrograph of frozen section of liver obtained from one of the two atypical animals which received 5 daily injections of the extract. The preparation was stained with Scharlach R. The liver cells contain large numbers of fat globules which are dark in the photograph. The fat in the epithelium of the bile ducts is also apparent. (This is normal in the larger ducts.) In this animal there was degranulation of the alpha cells of the pancreas. This picture illustrates the probable liver-fat-depositing effect of the extract.

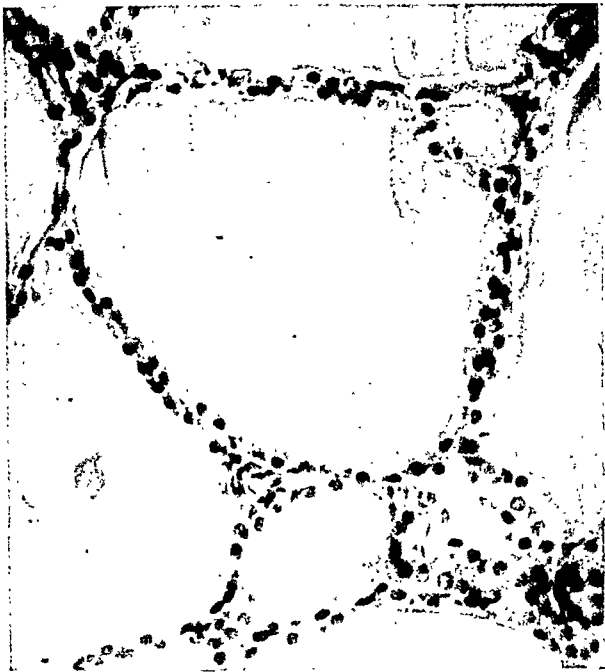
FIG. 16. Oil-immersion photomicrograph of a section, stained by haematoxylin and eosin, of the parathyroid gland of a dog which received 4 daily injections of the extract. A mitotic figure may be seen in the centre of the picture. This picture illustrates the parathyrotropic effect of the extract.

permanently diabetic animals which we studied appeared atrophic as compared to normal ducts. It is possible that after a long period of unproductive stimulation from the animal's own pituitary gland, the character and potentiality of these stem cells might alter.

We do not believe that new beta cells can form from fully differentiated acinar cells, despite the recent support this theory has received from Woerner,³⁰ Sergeyeva³¹ and Richardson.¹⁶ Bensley¹¹ reviewed this problem several years ago. He found no convincing evidence that acinar cells could form islet cells and postulated certain criteria which would have to be satisfied before such a premise could be granted. One set of requirements was that cells should be found which contained the granules of both acinar and islet cells. These requirements have been met, according to Woerner, Sergeyeva and Richardson. We too, in this study, occasionally observed cells which appeared to fulfill these requirements, but it is very difficult to be certain about observations of this nature. Even if such cells exist in the pancreas, we think that they offer no evidence with regard to acinar cells becoming islet cells or *vice versa*. From studies made by one of us³² on the formation of cartilage and bone, it became apparent that when stem cells exist and retain equal potentiality for differentiating into two end-products, the path which differentiation takes depends upon environment. When the environmental influence is not clear-cut, the stem cells differentiate along a middle course and produce tissues having the characteristics of both of the usual end-products. Hence we regard these cells with the characteristics of both acinar and islet cells as nothing more than an indication that stem cells in this case differentiated in a complex environment. Gradation tissues, in our opinion, indicate metamorphosis only when they exist between a series of cells in progressive stages of differentiation. We consider acinar cells and beta cells both as highly differentiated elements.

SUMMARY

Histological studies were made on the organs of a large number of dogs made diabetic with an anterior pituitary extract by Best, Campbell and Haist, who have reported on aspects of diabetes in these dogs other than the histopathology.



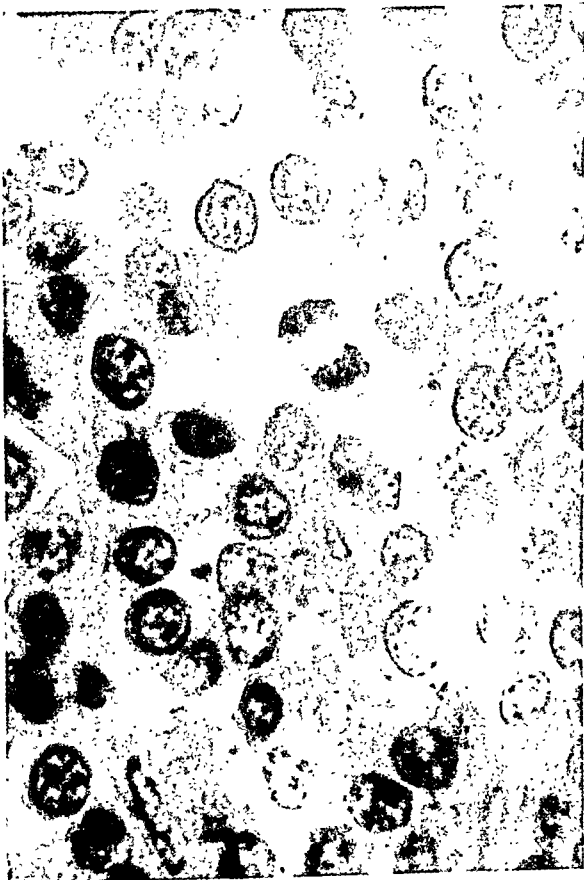
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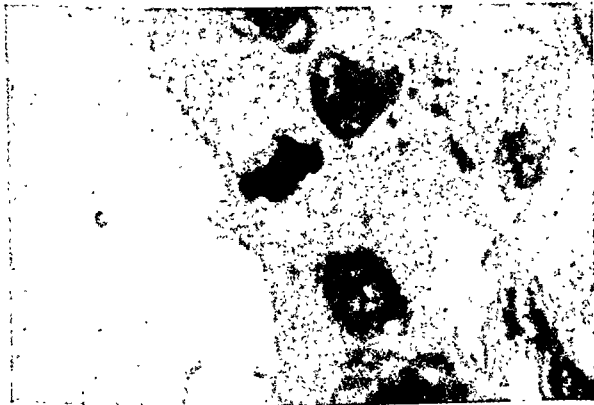
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Daily injections of this extract led to a progressive degranulation of the beta cells of the islets of Langerhans. This was usually almost complete after 7 daily injections. The later stages of degranulation were associated with the development of hydropic degeneration of the beta cells which became very extensive immediately following degranulation. The cells of the smaller ducts and cords of undifferentiated epithelium also became hydropic.

During the first 7 days of injections, hypertrophy, hyperplasia and cytoplasmic changes indicative of increased secretory activity developed in the cells of the thyroid gland, the parathyroid gland and the adrenal cortex. During this same period many mitotic figures were observed in the cells of pancreatic acini, in the cells of the small ducts of the pancreas and in the cords of epithelial cells of the pancreas. Before hydropic degeneration was severe, mitotic figures were also seen in pancreatic islets.

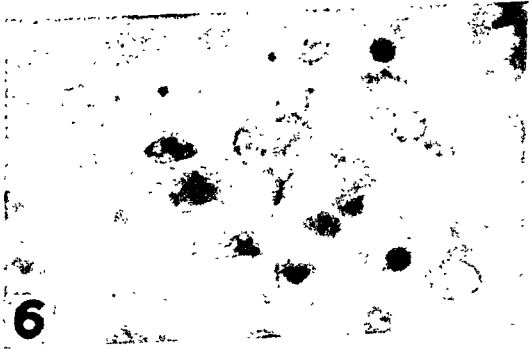
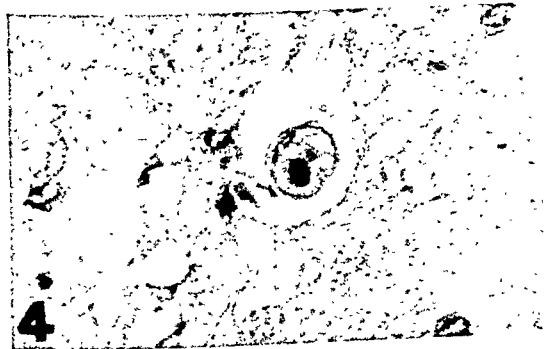
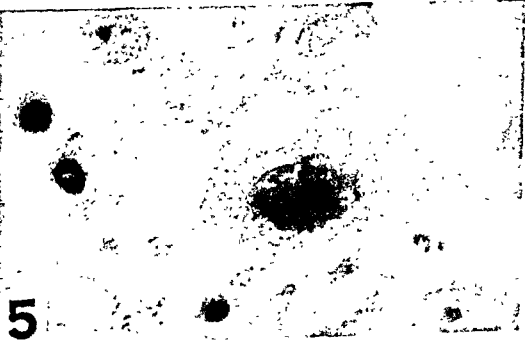
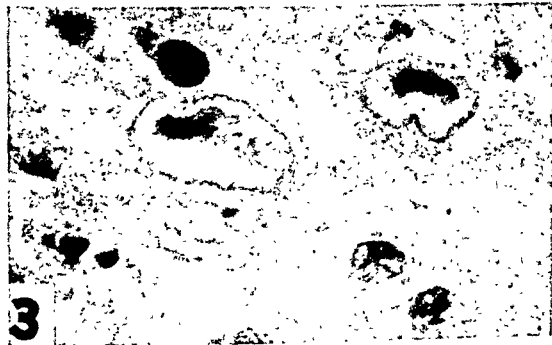
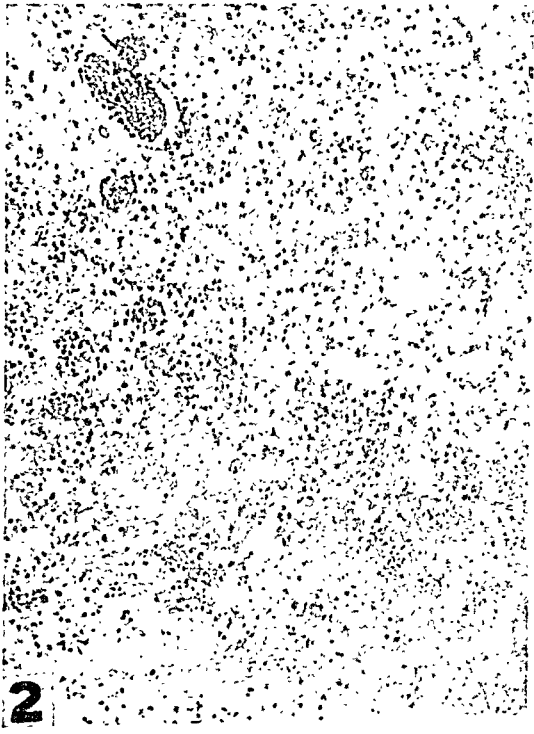
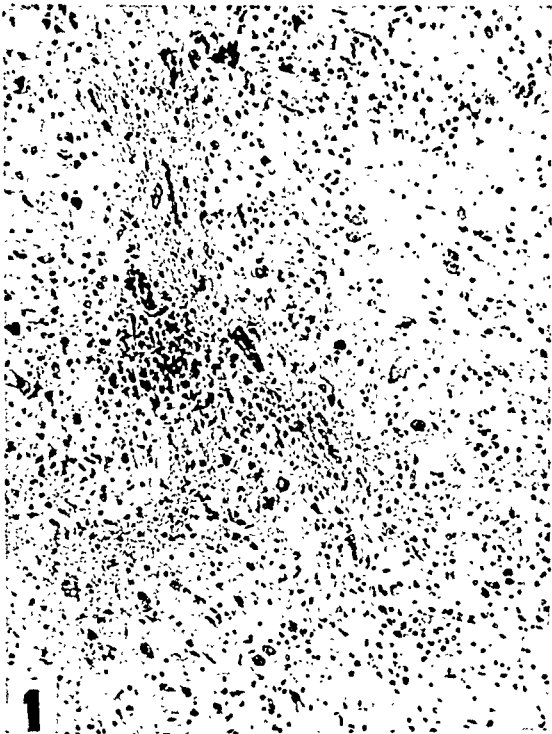
After 3 daily injections of extract most injected animals showed areas of hydropic liver cells. In these cells the granular cytoplasm was depleted and the cells were swollen with fluid which contained considerable glycogen. In some animals injected for 7 days, more than one-half of the liver cells were affected in this manner. Most of the livers of the injected animals demonstrated very little fat in frozen sections. In 2 animals injected for 5 and 7 days, the livers were very fatty. In 1 of these animals the alpha cells of the pancreas were degranulated; in the other animal the pancreas evidenced some depletion of alpha granules but degranulation was not complete. In all other animals the alpha cells were well granulated.

In a series of animals allowed to recover after receiving 7 daily injections of extract, the beta cells had recovered about one-half of their granules 3 days after the last injection. Four days after the last injection granulation was complete. Three days after the last injection, recovery had occurred from such hydropic degeneration as was present after 7 daily injections. The connective tissue of the islets was increased in animals which recovered after receiving 7 daily injections of extract.

In 3 animals which were injected with daily doses of extract for 21 to 30 days and from which pancreases were obtained months later there was a scarcity of islets and a lack of granular beta cells.

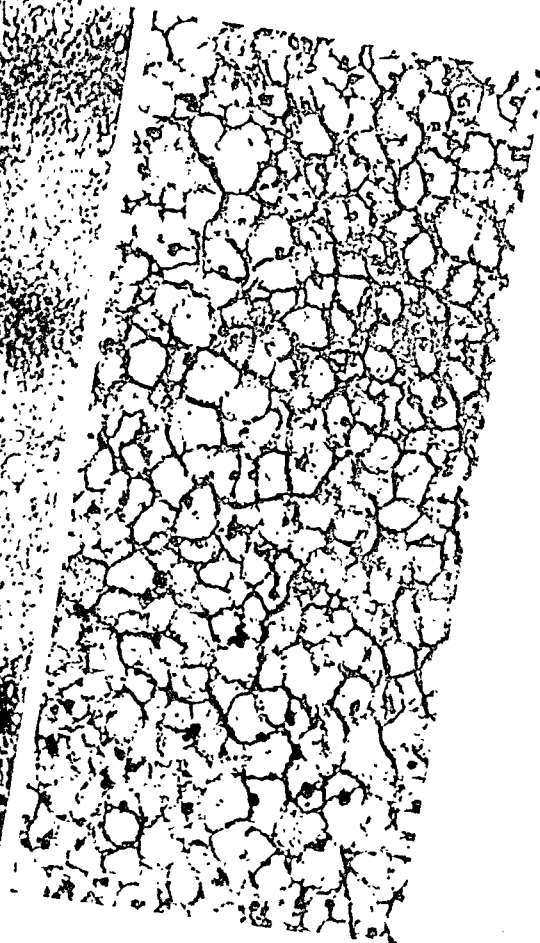
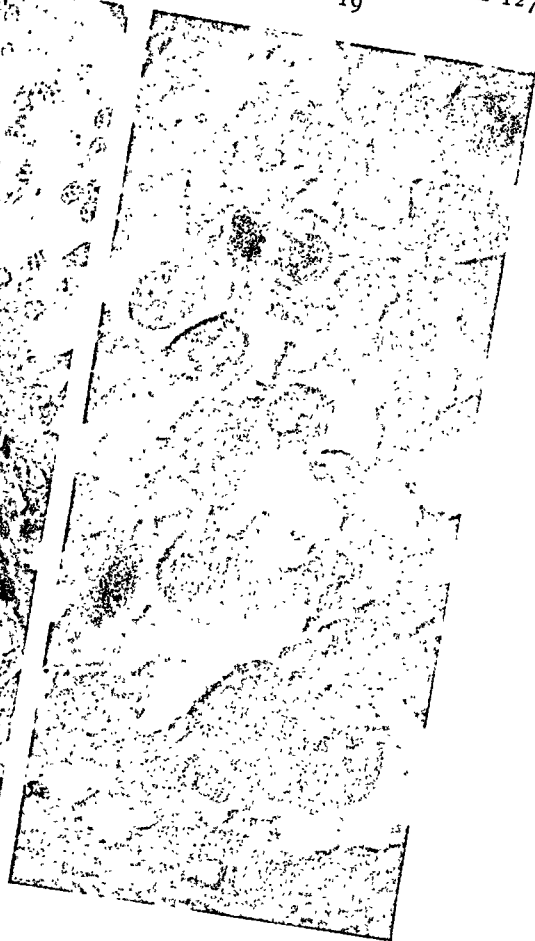
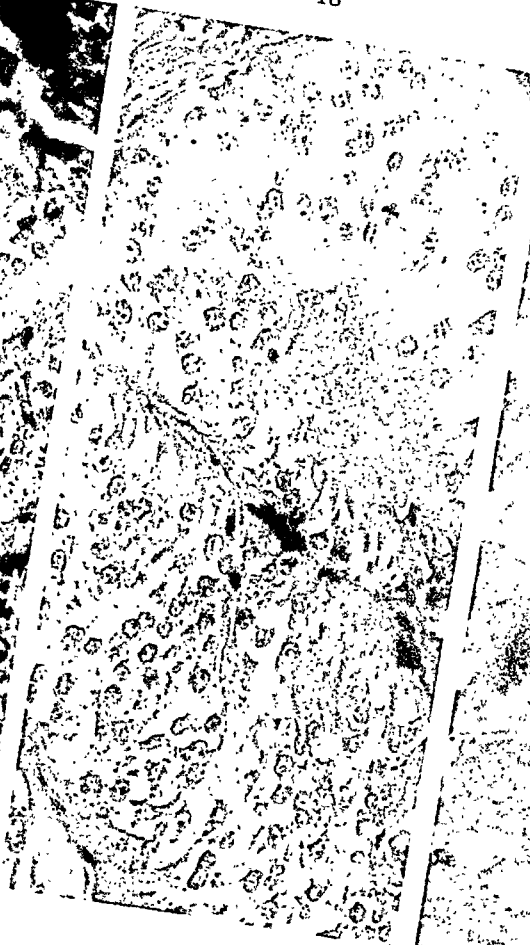
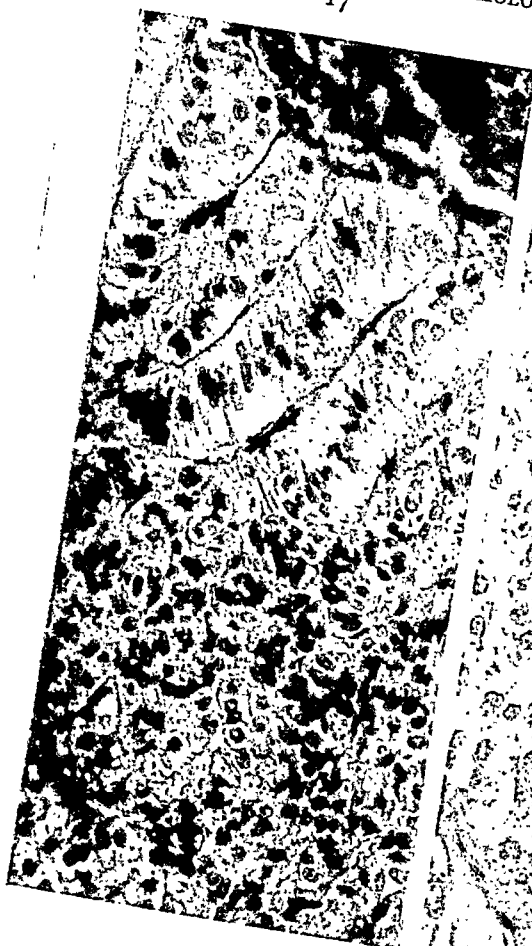
PLATE 127

- FIG. 17. Medium power photomicrograph of a section of the adrenal cortex from a control dog. The characteristic zona glomerulosa appears under the capsule at the top of the picture.
- FIG. 18. Photomicrograph of widened zona glomerulosa and cellular-hypertrophy observed when a dog is given 4 daily injections of extract. Magnification is the same as for Figure 17.
- FIG. 19. Oil-immersion photomicrograph of zona glomerulosa of a dog given 4 daily injections of extract. Two mitotic figures as well as extensive cell vacuolation and cell hypertrophy are obvious. This, and Figure 18, illustrate the adrenocorticotropic effect of the extract.
- FIG. 20. Low power photomicrograph of a section stained by haematoxylin and eosin of liver obtained from an animal which received 3 daily injections of the extract. The liver cells near the central vein are extremely well filled with glycogen, causing the vacuolation observed.
- FIG. 21. Very low power photomicrograph of liver of an animal which received 7 daily injections of the extract. The liver cells of the greater part of each lobule are distended with clear fluid which contains glycogen.
- FIG. 22. High power photomicrograph of an area selected from the section shown in Figure 21. There is little granular cytoplasm in these cells; the nuclei are frequently pyknotic and at one side of the cell. Glycogen stains showed that the cells contained considerable glycogen.



Smith, Lennette and Reames

Herpes Simplex and Acute Encephalitis



Ham and Haist

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Histopathogenesis of Diabetes

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16. Knapp, Arnold. Bilateral glioma. Report of a case unsuccessfully treated with radium. *Arch. Ophth.*, 1920, 49, 574-584.

FIG. 23. Oil-immersion photomicrograph of a section stained by the Bowie method, of pancreas obtained from a dog 2 days after the last of 7 daily injections. One alpha cell can be seen to the left of the centre of the islet. The beta cells do not contain their normal granulation, the cytoplasm of these cells consisting only of the pale background in which the specific granules can usually be seen.

FIG. 24. Oil-immersion photomicrograph of a Bowie preparation of a pancreas obtained from a dog 3 days after the last of 7 daily injections. Three alpha cells with their large dark-staining granules can be seen on the left side of the islet. The beta cells which comprise the remainder of the islet show a returning granulation which is as yet not complete.

FIG. 25. Oil-immersion photomicrograph of an islet in a Bowie preparation of a pancreas obtained 4 days after the last of 7 daily injections of the extract. Beta cell granulation is approximately normal.

FIG. 26. Oil-immersion photomicrograph of a Mallory-azan preparation of an islet from an animal in permanent diabetes. Almost all the cells in the islet contained red granules and are alpha cells. In the photograph their cytoplasm is light. The reticulum in and about this islet is increased over normal.

FIG. 27. Oil-immersion photomicrograph of a Bowie preparation from the pancreas of a permanently diabetic dog. The cells of this islet can be identified as alpha cells by their large dark-staining granules.

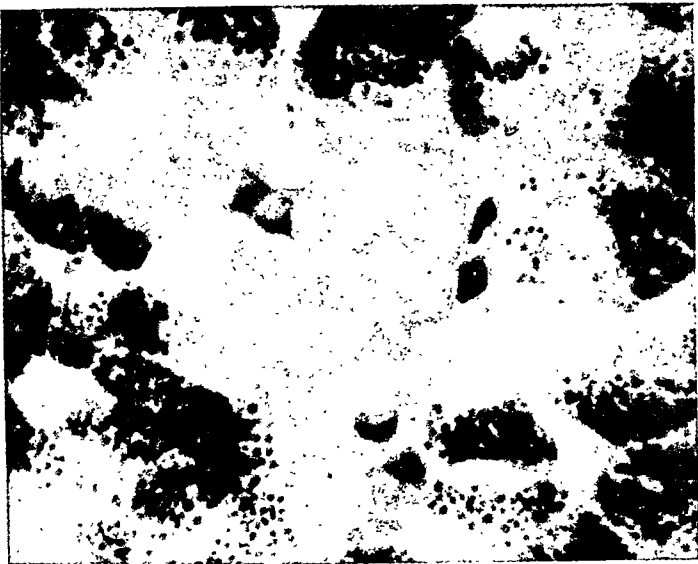
FIG. 28. High power photomicrograph of an area of fibrosis bearing a superficial resemblance to an islet seen in a section stained by haematoxylin and eosin of the pancreas of one of the permanently diabetic dogs. This was the closest approach to an islet that was found in this pancreas.

DESCRIPTION OF PLATES

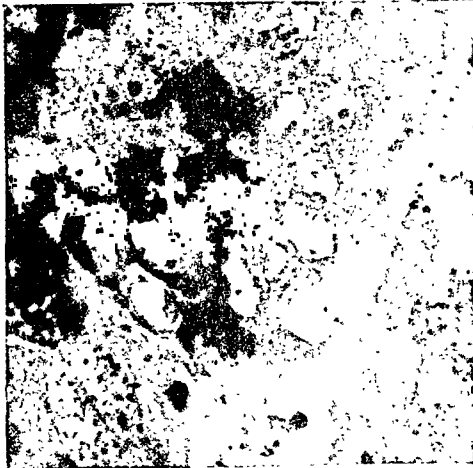
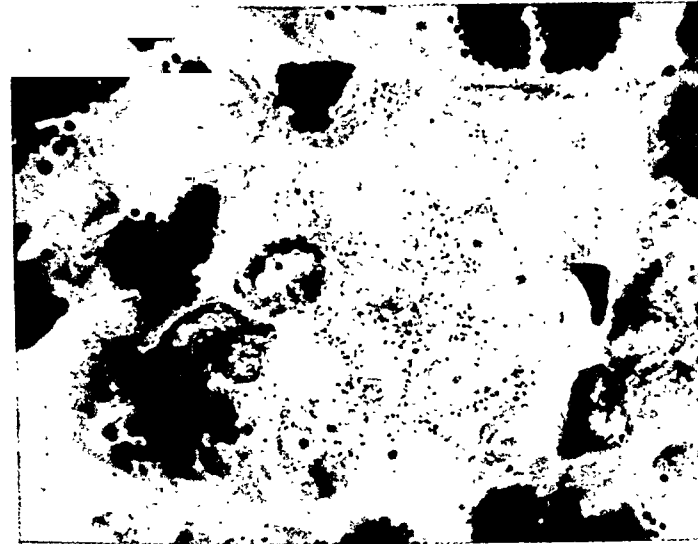
PLATE 129

FIG. 1. Horizontal section through the head and eyes, slightly below the level of the pupils. The right eye has been entirely replaced by dark tumor tissue but the sclerotic coat is still preserved. A large tumor mass in the right temporal region has partly destroyed and partly surrounded the right frontal bone, and has extended posteriorly and subperiosteally to the right temporal bone, and medially to destroy the posterior portion of the ethmoid bone. The tumor mass seen in the left temporal region was continuous with the right temporal mass at a higher level. The left eye showed one whitish nodule on the posterior segment of the retina and another seen in profile as a whitish band just behind the ora serrata. 7/10 natural size.

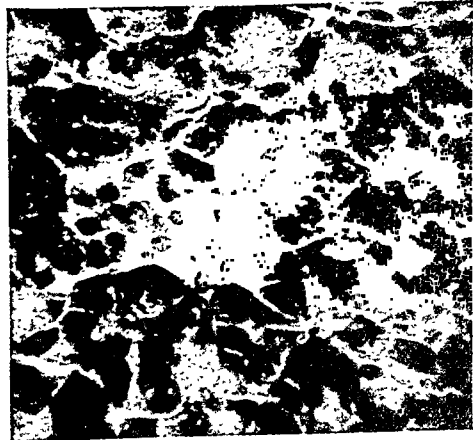
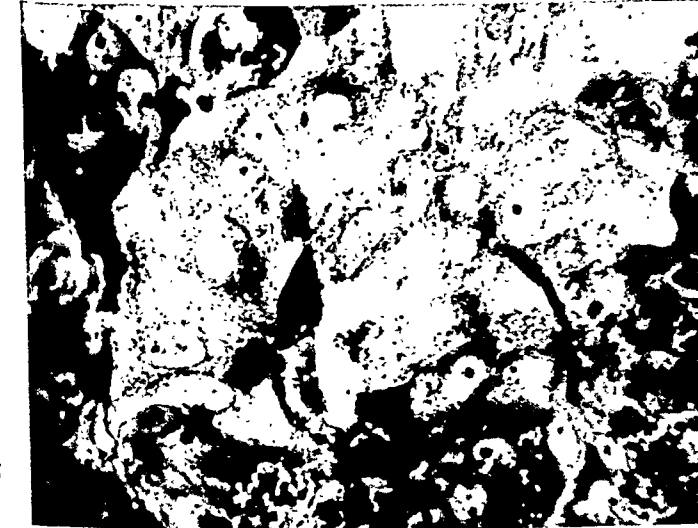
FIG. 2. Drawing of the sectioned left eye, showing the two whitish nodules of the retina, one just behind the ora serrata and the other in the posterior segment, viewed from a slightly different angle. The third nodule referred to in the text was on the opposite half of the eyeball and therefore could not be shown in this picture. Natural size.



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Ham and Haist

Histopathogenesis of Diabetes



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Histogenesis of Glioma Retinae

Ch'in

FIG. 3. Low power view of the smallest nodule of the left retina. At the right and left margins of the picture, the normal retinal layers are intact, while in the central portion the inner nuclear layer widens into a tumor nodule. The nodule bulges more on the inner surface (below) of the retina, where it is, however, still completely enveloped by the inner limiting membrane. On the outer surface (above), the outer nuclear layer can be seen to cover the nodule completely except at one area midway between the left margin and the middle line of the nodule. The deeper retinal layers are continued, on both the inner and outer surfaces, for only a short distance over the periphery of the nodule. $\times 20$.

FIG. 4. Peripheral portion of the nodule with the adjacent normal retina. From above downward can be seen the layer of rods and cones (gray in color, arranged as a palisade and somewhat torn by artefact), the outer limiting layer (a gray, somewhat wavy membrane), the outer nuclear (granular) layer (very broad and consisting of dark nuclei), the outer reticular (molecular) layer (pale), the inner nuclear (granular) layer (much narrower than the outer nuclear layer), the inner reticular (molecular) layer (much broader than the outer reticular layer), the layer of ganglion (nerve) cells (very thin, only two or three cells thick), the layer of nerve fibers (pale and broad) and the inner limiting membrane (as a sharp line). The cells of the nodule can be seen to arise clearly from the inner nuclear layer which gradually increases in thickness and passes imperceptibly into the nodule. The various retinal layers are seen to continue over the peripheral portion of the nodule. $\times 155$.

FIG. 5. Portion of retina, 5 mm. away from the nodule illustrated in Figure 3, showing a microscopic nodular swelling of the inner nuclear layer. $\times 155$.

FIG. 6. Central portion of the nodule illustrated in Figure 3, and its outer surface, showing the arrangement of the tumor cells in pseudorosettes. At the right margin of the figure can be seen the rods and cones, the outer limiting membrane and the persisting outer nuclear layer. $\times 220$.

THE HISTOGENESIS OF GLIOMA RETINAE *

REPORT OF EARLY CASE WITH REVIEW OF LITERATURE

K. Y. CH'IN, M.D.

(From the Department of Pathology, Peiping Union Medical College,
Peking, China)

Glioma of the retina (retinoblastoma) is a rapidly progressing disease. At the time when the patient consents to operative removal, the condition is already anatomically far advanced and the histogenesis of the neoplasm cannot be determined. Operative and necropsy material rarely offers an opportunity for the study of the derivation of the tumor and in the literature there are recorded relatively few cases of early tumor whose point of origin from the retina can be definitely traced. For this reason the following case is reported.

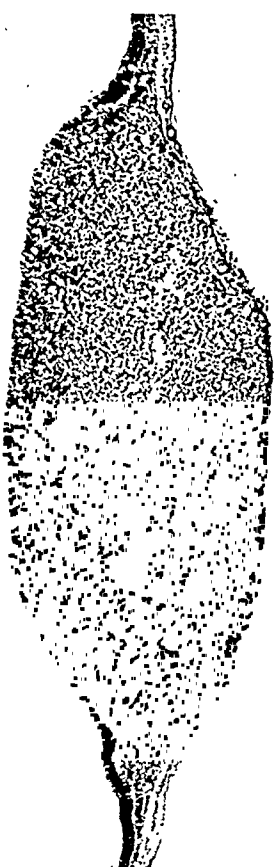
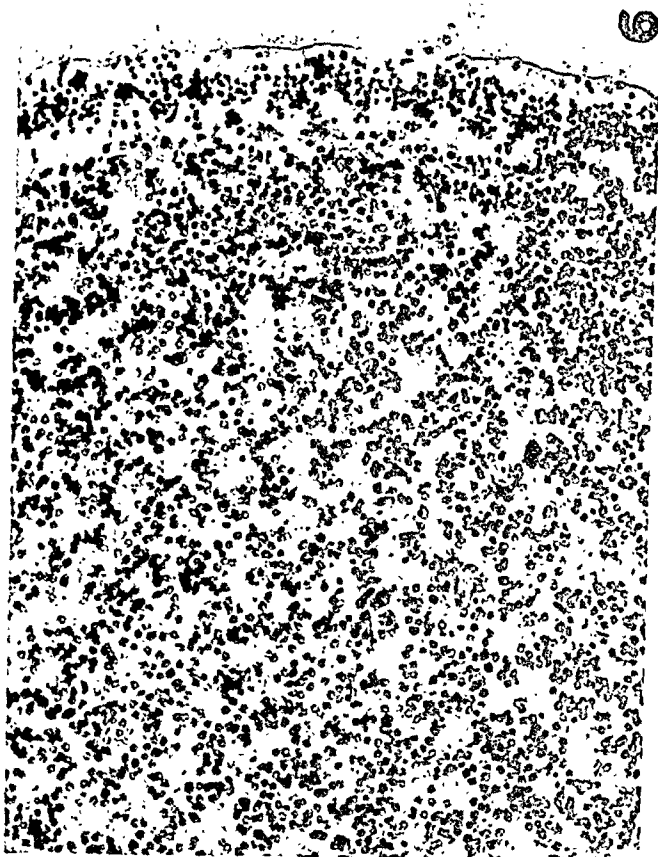
REPORT OF CASE

Clinical History

A Chinese female infant, 21 months of age, was admitted with a rapidly progressive bulging of the right eye, present 20 days prior to admission. At the age of 1 month, a yellowish white, round spot was seen in the center of the right pupil, which persisted without any change in size. At the age of 11 months redness of the bulbar conjunctiva and redness and swelling of the eyelids on the right side were noticed but gradually subsided. Twenty days before admission redness recurred in the right eye, soon followed by protrusion of the eyeball and then of the right temporal region. The bulging increased steadily and the patient began to complain of pain in the temporal region 10 days before entry. The white spot in the cornea became ulcerated and there was some bleeding from it in the last 5 days.

Examination showed marked enlargement of the right eyeball with pronounced exophthalmos and lagophthalmos. The conjunctiva was congested and swollen and the cornea was totally destroyed and represented by a small, dry, dark stump. The eyeball was hard and fixed. A hard, protruding mass was present over the right temporal region lying deeply and apparently connected with the bone. The left eyeball was normal externally. Examination of the left fundus showed several well circumscribed, whitish masses. These masses were followed by repeated ophthalmoscopic examinations and were found to be stationary in size. Needle biopsy of the right temporal mass showed a round-celled malignant tumor, a questionable neuro-epithelioma.

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After admission, two exposures of deep X-ray therapy were given to the right eye and temporal region. The tumor became, however, steadily larger, with much bleeding from the right eyeball. Five weeks later, a tumor mass was noticed over the left lower jaw as well as extension of orbital growth into the oral cavity with ulceration of the buccal mucosa. The skull also showed signs of involvement by the tumor. The patient became drowsy and died in a very cachectic state 8 weeks after admission.

Postmortem Examination

The tumor of the right eye was far advanced. It had destroyed the entire eyeball except for the sclerotic coat, which was only partly preserved. The tumor had perforated this coat and spread widely by direct extension. On the right side, a huge tumor mass was found in the temporal region, and the orbital wall of the frontal bone, the great wing of the sphenoid and the anterior portion of the temporal bone were destroyed by it. Subperiosteal extension to the parietal and occipital bones was found. The tumor had spread medially and destroyed the ethmoid bone and filled the ethmoid sinuses. It had also spread upward, invading the cranial cavity and pushing the dura upward, and had spread to the opposite side to form a large mass in the left temporal region with subperiosteal extension to the left petrous and parietal bones. The brain showed marked compression and pressure atrophy of the right frontal, temporal and parietal regions. The tumor mass in the temporal region on both sides spread downward so as to destroy both maxillae (eroding the hard palate) and both sides of the mandible (filling the alveolar sockets). The tumor also had spread to the soft tissues of the right cheek and neck. Metastases were found in right eighth, ninth and tenth and left seventh and ninth ribs, femora, tibiae, fibulae, humeri, liver, pancreas and muscle of the right thigh.

The interesting finding in this case concerned the opposite (left) eye. The retina showed three well circumscribed, whitish nodules, circular and somewhat flattened in shape. They measured 4, 5 and 6 mm. in diameter, respectively, and were elevated above the inner retinal surface for 1 to 2 mm. One of them was situated immediately behind the ora serrata and the other two in the posterior segment of the retina behind the equator. The retina could easily be lifted away from the chorioid because these nodules had not invaded the latter coat. When both sides

of the retina were examined, the nodules were found to present a smooth surface on both sides and appeared to be confined within the limiting membranes of the retina. They bulged to a greater degree on the inner surface.

The smallest nodule was examined microscopically. It was found to be confined within the limiting membranes of the retina which were still intact. All of the retinal layers were normal up to the very margin of the nodule. At this point, the inner nuclear (granular) layer thickened and passed imperceptibly into the nodule. The cells of the nodule were small and round and had vesicular nuclei with fine chromatin granules and scanty cytoplasm and were identical in appearance with the cells of the inner nuclear (granular) layer except (1) that the former were hypertrophic with larger nucleus and a small amount of visible acidophilic cytoplasm (which was absent in the latter) and showed marked variation in the size of the cells, and (2) that the cells in the interior of the nodule were arranged in pseudo-rosettes. No true rosettes lined by columnar cells were present.

The nodule bulged more on the inner surface of the retina and therefore pressed upon or permeated into the inner layers of the retina much more than the outer layers. Thus the layer of nerve fibers, the ganglion cell layer (layer of nerve cells) and the inner reticular (molecular) layer were continued only for a short distance over the periphery of the nodule, while over the convexity of the nodule they were entirely destroyed and replaced by the cells of the nodule which was, however, throughout covered by the still intact inner limiting membrane. On the outer surface, where the nodule bulged only slightly, the outer reticular (molecular) layer was continued for some distance over the periphery of the nodule and then became lost over its convexity, while the outer nuclear (granular) layer, the outer limiting membrane and the layer of rods and cones were pushed outward with the outer nuclear layer moderately compressed and thinned out but were still intact throughout except at one spot where these layers had been destroyed and replaced by the tumor (nodule) cells which lined the free surface.

Another interesting finding was that at a distance of 5 mm. from this nodule, the inner nuclear layer showed another nodular thickening or hyperplasia, which was so small that it could be

CARCINOMA OF CERUMINOUS GLAND *

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We report one case of carcinoma of ceruminous gland. Judging from our own experience and from the literature this is one of the least common tumors.

REPORT OF CASE

A male, 78 years old, over a period of 1 year, developed a subcutaneous tumor in the hollow behind the right ear which was bounded by the lobule, lower auricle and mastoid process and increased more rapidly in the latter part of the year. Right lower facial paralysis developed 6 months after the tumor was noticed. Latterly the slightly elevated eminence formed by the tumor became ulcerated. The tumor was excised by Dr. C. C. Lund by wide dissection, including the posterior tip of the parotid and the deeper muscles and extending to the skin of the inferior aspect of the external auditory meatus. A complete right facial paralysis followed the operation.

Gross Description

The specimen consisted of an oval mass of tissue with skin on one surface, measuring 4 by 2.5 cm., with underlying tissue 2 cm. in thickness. The skin surface was smooth and not remarkable except for a sharply outlined ulcer, 1.2 cm. in diameter, in the center. Yellowish brown, granular tissue was present in the ulcer. The specimen as a whole was extremely firm. The cut surface showed juicy, resilient tissue at the ulcer and for about 1 cm. under the surrounding normal skin. One cm. from the skin surface the superficial tumor merged with a scirrhous, irregularly outlined mass 2 cm. in diameter. Cysts containing slightly yellowish, granular fluid, varying from 0.2 to 0.6 cm., were present in this more dense tissue. The facial nerve emerged from the edge of this mass. At one end of the specimen a small portion of salivary gland was recognized.

Microscopic Examination

Microscopic sections showed an adenocarcinoma varying markedly in degree of differentiation, the most anaplastic portions being at the ulcerated surface of the skin. Here the tumor had

* Received for publication April 11, 1941.

recognized only microscopically. The histological picture of this nodule differed from that of the grossly visible nodule only in that hypertrophy of the individual cells and pseudorosette arrangement were not appreciable.

DISCUSSION

Gliomas of the retina arise mainly from the ciliary portion (*pars ciliaris retinae*) and the posterior segment of the retina (Ewing¹). According to Wintersteiner,² the origin is often multiple and chiefly from the posterior pole, while Eisenlohr³ stated that the ciliary portion is the site of predilection and that the frequency of origin from it as compared to origin from the posterior segment is 11 to 7.

Grossly, multiple early gliomata have been described ophthalmologically by Hirschberg and Happe⁴ and Hirschberg⁵ as giving the appearance of acute miliary tuberculosis of a serous membrane. Multiple focal opacities, semiglobular elevations and small nodules have also been noted. Microscopically, glioma retinae may arise from the inner layers of the retina and invade and be situated mainly in the vitreous humor, known as glioma endophytum; or it may arise from the outer retinal layers and be situated mainly between the retina and the chorioid, known as glioma exophytum. Hirschberg and Happe⁴ described an early tumor arising as circumscribed collections of round cells in the inner nuclear layer, which they considered to be the common origin for this tumor. Flexner⁶ described a fair-sized tumor which, although connected with the retina throughout a considerable part of its extent, was seen to originate at a point of microscopic size situated in the external nuclear layer. Schweigger⁷ observed also a tumor arising as a localized hyperplasia of the cells of this layer. Eisenlohr³ stated that the tumor at times arises from the inner, and at times from the external nuclear layer. Iwanoff's⁸ early tumor was in the form of an interstitial infiltration of the nerve fiber layer by small round cells even without causing thickening of that layer. Leber⁹ stated that the fibrillar (reticular) layers of the retina can also be the starting point of gliomas. Delafield¹⁰ described a tumor mainly involving the nerve fiber layer and inner nuclear layer and thought that it could arise anywhere inside the outer reticular layer. Knapp¹¹

no uniform architecture, but grew in irregular strands and clumps with little intervening corium. There was no connection with the overlying skin. The cells were large and polyhedral with a finely granular or foamy cytoplasm which tended to be neutrophilic. In size the nuclei were moderate to large. Mitotic figures, some of which were atypical, were not as numerous as one might expect from the degree of anaplasia. At one point the tumor had extended slightly into a lobule of parotid gland. But in the scirrhous part of the tumor the cell structure became more differentiated and a distinct architectural pattern was obvious. The cells were smaller and arranged in alveoli, some of which were distended with secretion and débris to form minute cysts. In one part of the tumor near the under surface of the specimen, and at the opposite end from the parotid, the structure was so well differentiated that in a given field it was impossible to be sure whether the alveoli were an adult form of the tumor or were glands surrounded by tumor, so closely did they resemble the normal ceruminous gland. These alveoli, each about twice the size of an alveolus of a sweat gland, were formed of tall cuboidal to columnar cells having the characteristic lipped margins of the ceruminous and apocrine glands and a rather small nucleus placed in the center of the cell or slightly toward the basement membrane. One or two spindle-shaped nuclei, which might be either in smooth muscle cells or in fibrocytes, partly encircled the glands. The typical myo-epithelial cells of the sweat glands and also occasionally of the ceruminous gland were not seen.

The most interesting feature of the tumor cells in the deep scirrhous portion, allying them to the superficial anaplastic tumor, was the reticulated, foamy and finely granular cytoplasm. Fine fat droplets could be stained with Sudan IV. In a majority of the cells, the granules had the same neutrophilic tint as the cytoplasm as a whole, but in many the granules were much larger, stained a deep blue and were clumped at the free end of the cell or around the nucleus. In about one-fourth of the glands there were amber granules in the cytoplasm. Many of the granules gave a positive iron reaction. The epithelium lining the cystic alveoli was less uniform; in some places it was flattened, in others many-layered and in still others there were miniature papillary cystadenomas. Cellular débris, blood and amber extracellular pigment granules

found the inner granular (nuclear) layer to be the starting point of the bulk of a tumor but at the same time observed multiple foci of neoplastic proliferation in the outer nuclear layer and in the inner (reticular, ganglionic and nerve fiber) layers of the retina. The retina was two or three times its normal thickness and the normal elements were destroyed and replaced by tumor cells but both limiting membranes were intact and only pushed aside. Thus Iwanoff,⁸ Jackson¹² and Stout¹³ have concluded that the tumor can arise from any or all of the layers of the retina.

Jokl¹⁴ described an early tumor situated in the center of the optic nerve head and found the tumor cells to be identical in appearance, and also continuous, with the cells of a tongue-like process of glial tissue around the hyaloid artery. Since Seefelder¹⁵ had described a migration of glial cells from the optic papilla in a fetus of 7 months' intra-uterine life to form a tumor-like mass of glial tissue around the hyaloid artery and his own observation had shown a direct continuity between the tumor and glial tissue around the artery, Jokl considered such glial tissue the origin of the neoplasm and viewed the tumor in the light of a congenital malformation. He further supported his view by referring to the coexistence of this tumor with such malformations as microphthalmos, coloboma and pupillary membrane as reported in the literature. The fetal or congenital origin of glioma retinae has also been emphasized by Eisenlohr.³

The case herein reported is one of bilateral glioma retinae, in which the tumor on one side was far advanced with widespread local extension and distant metastases while the tumor on the other side was at a very early stage, still confined within the retina and with a clearly demonstrable origin from the inner nuclear layer. With a large tumor in the right eye, it is theoretically possible that the small nodules in the left eye were metastatic, but in favor of their primary origin are the following findings: (1) the gradual thickening of the inner nuclear layer and its gradual passage into the nodule without a perceptible margin of transition (Figs. 3 and 4); (2) the microscopic nodular swelling of the inner nuclear layer elsewhere (Fig. 5); (3) that the nodule of the left eye showed a histological picture different from that of either the primary tumor of the right eye or its

were present in many of the cysts. It seemed rather curious that those well formed glands were lying in perivascular lymphatics and penetrating nerve trunks whereas the less differentiated tumor was not seen in either lymphatics or blood vessels. There was no evidence of gross metastasis.

DISCUSSION

The differential diagnosis lies between carcinoma of an apocrine gland and carcinoma of a ceruminous gland. Both are modified coil or sweat glands. Their normal histology is similar. The chief points of difference are in the rather more conspicuous pigment and lipid in the ceruminous gland, which at best is a rough quantitative distinction, and the more conspicuous smooth muscle which sometimes surrounds the ceruminous glands.

These histologic variants are of no practical interest in this case for the following reasons:

(1) Judging from the degree of differentiation of the various parts of the tumor with which we are concerned, it originated in the deep portion of the specimen near the auditory canal.

(2) Apocrine glands rarely, if ever, occur in this region.

(3) Only the apocrine glands of the axilla or genital region contain readily demonstrable lipid or pigment.

From the paucity of reports of tumors of the ceruminous gland in the literature it is fair to assume that this is an extraordinarily rare tumor. Montpellier and Laffargue¹ in 1938 published a careful and convincing report of carcinoma of the ceruminous gland with very good illustrations. As in our tumor, there were very undifferentiated portions. The authors had seen a similar tumor in the ear of a cat. Adenomas of the ceruminous gland, reported by Brock² and by Sprenger and Prietzel,³ also appear correctly classified. In all of these cases the tumor appeared to originate from the intimately associated normal ceruminous gland.

Sprague⁴ in 1898 and Yearsley and Butterfield⁵ in 1924 reported a case with appearances suggestive of origin in the ceruminous gland. Only the probability of carcinoma of ceruminous gland was claimed by these authors. Sprague's tumor was highly invasive, infiltrating the skull and dura. Yearsley and Butterfield's case was apparently of a low degree of malignancy.

metastases. In the latter, the distinct pseudorosette arrangement of the tumor cells was absent, and the tumor cells were much larger, much more hyperchromatic and much more irregular in shape. This disease is bilateral, according to Ewing¹ in 23 per cent, and according to Knapp¹⁶ in 20 per cent, of those affected. The tumor is less advanced in the second eye, or may appear after an interval of 3, 4, or even 13 years (Knapp¹⁶) following the enucleation of the tumorous eye as new primary growth. In my case, the age (21 months) of the patient, the location of the early gliomata (in the posterior segment) and their multiplicity, the origin from the inner nuclear layer and the histological picture of a nodular hyperplasia of the cells of the nuclear layer are all typical for this disease, as a review of the literature has shown. The stationary size of the three nodules as found by repeated fundal examination bears out Ewing's statement that the initial growth of the tumor may be very slow.

It may be pointed out that in the early lesions of the retina herein described there is a certain amount of overlapping between "malformation" and "neoplasm." If the microscopic nodular swelling of the inner nuclear layer remains quiescent, it may justifiably be called malformation of the retina. This situation reminds one of another lesion of the nervous system, namely, tuberous sclerosis, which may remain quiescent as a malformation but may develop into a malignant neoplasm (glioblastoma multiforme).

SUMMARY

An early glioma retinae (retinoblastoma) with clearly demonstrable origin from the inner nuclear layer and still confined within the limiting membranes of the retina is described and the literature on early glioma retinae is reviewed with reference to the site of origin and histogenesis.

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The 3 positive and 2 probable cases of tumors of the ceruminous gland differ from ours in that they all presented in the external auditory canal, and all but 1 from the superior surface of the canal.

Tumors of glandular origin of the ear have been a subject of much interest because of their extreme rarity. Nearly all of them originate in the middle ear. Klingel⁶ in 1891 reported an adenoma of a sebaceous gland of the ear canal. Parrish⁷ in 1924 reported a highly malignant tumor of the external auditory meatus which eroded deeply into the skull and caused death. This was diagnosed as a squamous cell adenocarcinoma.

Newhart⁸ reviewed the literature from 1899 to 1917 and found 34 cases of carcinoma of the middle ear. One case of adenocarcinoma of the middle ear was found by Furstenberg⁹ among 40,000 admissions. Fraser¹⁰ found 15 cases of carcinoma of the external auditory meatus and middle ear among 6,605 "affections" of the ear, including 2 cases of "malignant sebaceous adenoma" from the external auditory meatus. Three cases of malignant tumor of the middle ear, among 35,000 cases seen from 1919 to 1931, were reported by Richter.¹¹

Robinson¹² reported that in a group of 212,000 clinic patients there were 19 cases of carcinoma of the external auditory canal and 5 arising from the middle ear. Alonso¹³ reported 24 cases of malignant tumor of the auricle, 5 of the mastoid region, 5 of the external auditory canal and 6 of the ear drum and middle ear.

In 1935 Schall¹⁴ reviewed the tumors at the Massachusetts Eye and Ear Infirmary and found no case of adenocarcinoma among some 90,040 pathological specimens from the ear. In his personal practice he had 1 case of adenocarcinoma involving the external auditory meatus and middle ear among 4 cases of carcinoma in this location. Thorell¹⁵ reported from the Radiumhemmet that there was no case of adenocarcinoma among 13 cases of malignancy of the middle ear. Lukens¹⁶ in 1936 reported 1 case of adenocarcinoma of the external auditory canal with some mucoid secretion which he considered of low malignancy only. He accepted only 4 previously reported cases of carcinoma of the external auditory canal: Fraser's 2 cases, Schall's case and Furstenberg's case.

It appears that carcinoma of the external auditory canal is

THE PRODUCTION OF NEURONAL INJURY AND NECROSIS WITH THE VIRUS OF POLIOMYELITIS IN RABBITS DURING INSULIN HYPOGLYCEMIA*

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While engaged in a clinical study of chronic hypoglycemia in patients of all ages I was impressed with the fact that several patients gave histories of attacks of poliomyelitis with residual paralysis. It occurred to me that a disturbance in carbohydrate metabolism could be a factor in susceptibility to infection with the virus of poliomyelitis, especially since, during hypoglycemia, cellular oxidation will be reduced. It has been demonstrated both in men¹ and dogs² that the oxygen uptake of the central nervous system falls during insulin hypoglycemia. Using the Barcroft-Warburg technic, various workers^{3,4,5} have shown that excised pieces of brain, peripheral nerve and meninges utilize less oxygen as the amount of glucose in the nutrient medium is reduced. Wortis⁵ also found that, weight for weight, the nervous tissues of the young in any species consume more oxygen than those of the adult and he concluded that the young are more vulnerable to hypoglycemia than the adult.

The rhesus monkey is very susceptible and the rabbit resistant to the virus of poliomyelitis. Without knowing the blood sugar range in these animals it was suspected that the blood sugar in the monkey reached lower levels than in the rabbit. These suspicions were found to have a basis in fact through the investigations of Jungeblut and Resnick,⁶ who studied glucose tolerance in monkeys, and du Vigneaud and Karr⁷ who studied glucose tolerance in rabbits. In the monkey, values were obtained as low as 50 mg. per 100 cc., whereas, in the rabbit, the blood sugar was never observed to fall below 100 mg. per 100 cc. Cori⁸ has stated that the blood sugar of mammals normally remains in the neighborhood of 100 mg. In twenty-five determinations made on fasting rabbits I have never observed the blood sugar to be below 100 mg. It was therefore concluded that the resistance of the

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rabbit might be associated with the fact that its blood sugar never fell below 100 mg. and that at this concentration cellular oxidation in the nervous system and in other organs would be maintained at such a level as to enable the cells to protect themselves against invasion by the virus. It was thought that if hypoglycemia were induced in the rabbit and the virus of poliomyelitis then injected, evidence of action by the virus might be obtained.

METHODS

Rabbits were fasted for 24 hours. A fasting blood sugar specimen was drawn and 0.6 to 0.8 unit protamine insulin per Kg. of body weight was injected subcutaneously. Such a dose usually depressed the blood sugar to around 45 mg. per 100 cc. in 1 to 3 hours and maintained hypoglycemia from 3 to 5 hours, and sometimes longer. Blood sugar specimens were drawn at various intervals from an ear vein and sugar estimated after the macro-method of Folin and Wu. If symptoms of severe hypoglycemia, such as muscular weakness, exophthalmos, restlessness, hyperexcitability and convulsions were noted, a small amount of 20 per cent glucose solution was injected intravenously or intraperitoneally. About an hour after the insulin injection, 0.4 cc. of a 30 per cent suspension of monkey cord virus was injected intracerebrally under novocain anesthesia. Rectal temperatures were taken at frequent intervals. During hypoglycemia the temperature may fall from 1 to 4 degrees, due, no doubt, to reduced bodily oxidation of glucose. Food was withheld after inoculation. Some rabbits received injections of insulin on successive days. Intracerebral injections of virus were not repeated. Rabbits receiving intranasal instillations of virus were prepared in a similar manner: three doses of 1 cc. of a 50 per cent suspension were instilled into each nostril every 2 hours on the first day and one dose daily for 1 to 3 days thereafter. Insulin was injected whenever nasal instillations were made on successive days. The virus used in these experiments was the MV strain of the Rockefeller Institute.

RESULTS

*I. Rabbits Injected with Monkey Cord Virus Suspensions.**

A. INTRACEREBRAL INJECTION. Four rabbits inoculated by

* In preparing virus suspensions from infected rabbits only the cord was used.

uncommon and adenocarcinoma extremely rare. Adenomas and carcinomas of the sebaceous glands of this region are reported occasionally, but a tumor having origin in a ceruminous gland is a curiosity.

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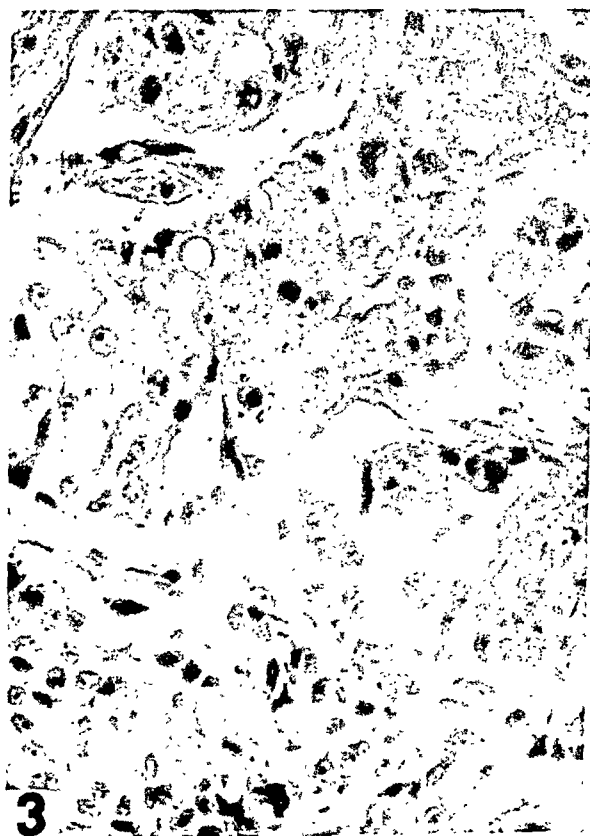
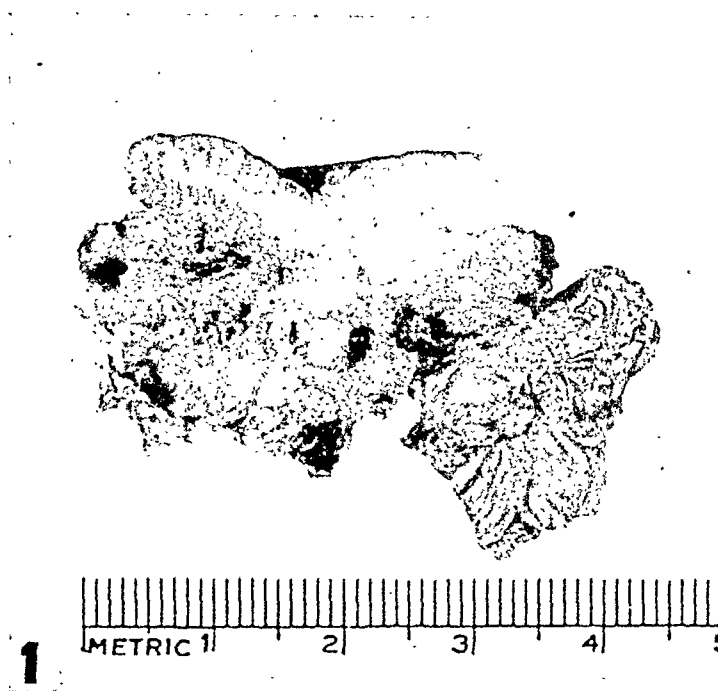
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DESCRIPTION OF PLATES

PLATE 131

- FIG. 1. Section through specimen removed, showing skin at upper border and extent of tumor.
- FIG. 2. Portion of tumor showing variation in size and character of epithelium. $\times 360$.
- FIG. 3. Portion of tumor showing epithelial masses and some vacuolated cells. $\times 360$.



THE RELATIVE SUSCEPTIBILITY OF THE SYNAPTIC TERMINALS AND OF THE PERIKARYON TO ARREST OF THE CIRCULATION OF THE BRAIN *

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The opinion is widely held that the synapse is more sensitive to lack of oxygen than is the cell body of the neuron.^{1,2} The evidence on which this hypothesis is based comes largely from anatomical studies of the vascularity of different portions of the nervous system.³

Recent studies do not support this view. Craigie⁴ noted that some areas rich in synapses are poor in capillaries. Bronk⁵ found that the synapse was not more sensitive to anoxia than the perikaryon in the stellate ganglion and Gerard⁶ observed that the apparent metabolism was higher in the perikaryon than in neuropil in the brain.

An opportunity presented itself to study the relative resistance of different portions of the neuron to arrest of the circulation of the brain. Studies were made of the morphological changes produced in the perikarya of the Purkinje cells of the cerebellum and in their associated synaptic terminals by equal arrest of circulation in the two adjacent structures. This investigation indicates that the synaptic terminals have a greater resistance to damage by anoxia than have the perikarya with which they are associated.

METHODS

The blood flow through the brain was stopped completely for periods of 2 to 8 minutes in adult dogs. The method of producing complete temporary arrest of the cephalic circulation by means of a cervical pressure cuff has been described elsewhere.⁷ Six animals were subjected to stasis in the brain and one served as a normal control. The brain was removed immediately after death and fixed

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PLATE 132

FIG. 4. Invasion of nerve by carcinoma of ceruminous gland. $\times 500$.

FIG. 5. Portion of tumor showing a neoplastic giant cell. $\times 320$.

FIG. 6. Portion of tumor showing lipping of tumor cells. $\times 510$.

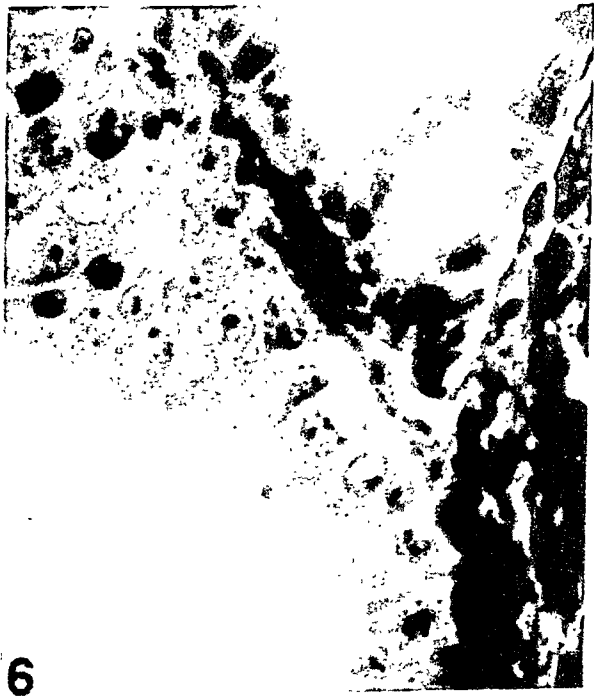
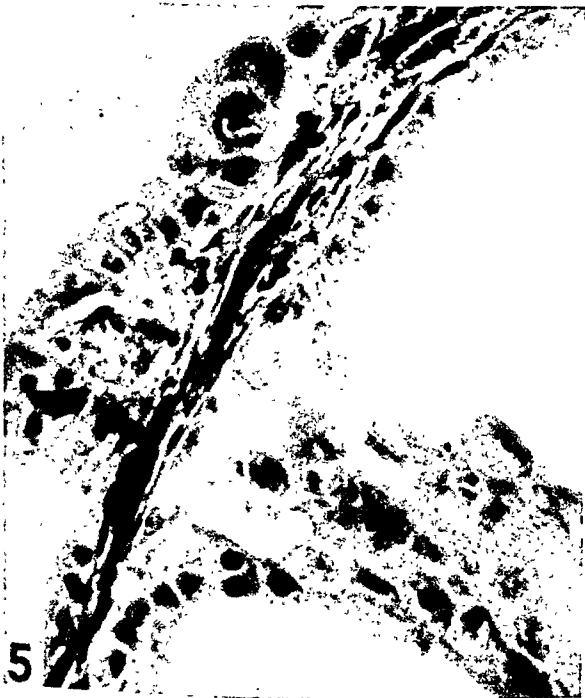
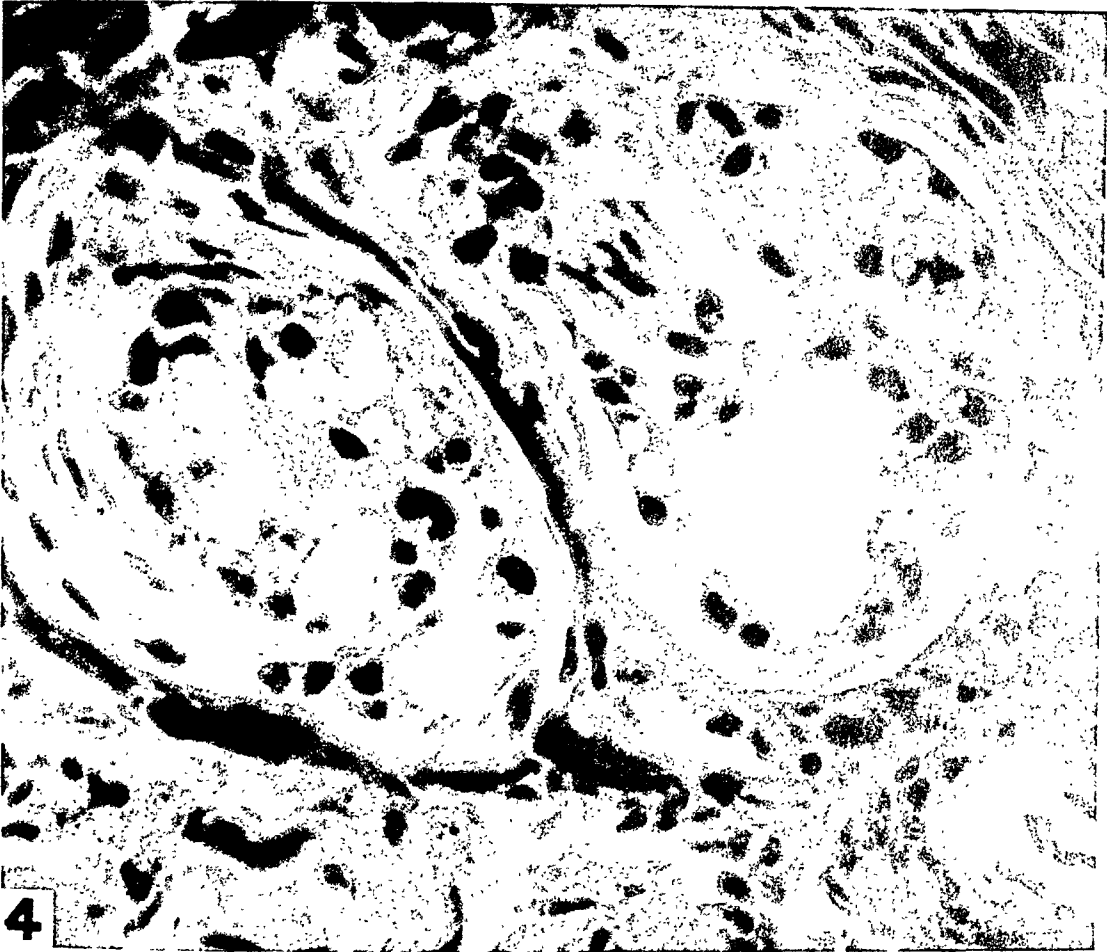
in formaldehyde. Portions of the cerebellum were dehydrated in dioxane, embedded in paraffin, sectioned at $8\ \mu$ and stained with hematoxylin and eosin, thionin and in some cases with the Bodian silver technic and phosphotungstic acid-hematoxylin. Other blocks from the same cerebellum were stained according to the block silver method of Cajal, modification 4a. The material stained by the Cajal method was embedded in paraffin and sectioned at $10\ \mu$.

RESULTS

The Purkinje cells of the cerebellar cortex are among the structures in the brain of the dog most sensitive to arrest of the circulation. These cells show pathological alterations after only 2 minutes of stasis, while after 8 minutes of arrest of the circulation of the brain almost all of them disintegrate and disappear. On the other hand, the other neurons of the cerebellar cortex are resistant to arrest of the circulation, since they remain morphologically normal after the Purkinje cells have disappeared (Fig. 1). The granule cells of the cerebellar cortex are even more resistant to stasis than are many nuclei of the brain stem. Sensitivity of Purkinje cells and resistance of other neurons of the cerebellar cortex to arrest of circulation has also been demonstrated in the cat.⁸ Physiological evidence also supports the view that the Purkinje cells are very sensitive to arrest of blood flow.^{9,10}

All of the types of boutons terminaux described by many authors¹¹ can be seen on the surface of the normal Purkinje cell (Fig. 2). These types include: simple terminal loops, filamented terminal loops, fibrillated bulbs and boutons-en-passant. Similar structures have been demonstrated in the experimental material to be described. Pathological changes in the boutons terminaux were never observed.

The synaptic terminals on the Purkinje cells originate in neurons of the spinal cord, brain stem and cerebellar cortex. The perikarya of the neurons in the spinal cord were not subjected to arrest of blood flow. The neurons of the brain stem which send their fibers to the Purkinje cells showed no significant pathological alterations as a result of the periods of stasis employed in this investigation. The neurons of the cerebellar cortex other than the Purkinje cells were also resistant to these periods of arrest of circulation. While some synaptic terminals arise as collaterals of



the Purkinje axons themselves, these are relatively few in number. Therefore (with the latter terminals as a minor exception) pathological alterations in the boutons terminaux on the Purkinje cells could occur only as an effect of arrest of blood flow on these boutons themselves.

Dog No. 3, a normal adult male, subjected to arrest of the circulation of the brain for 4 minutes, was sacrificed at the end of 3 months. In thionin-stained sections, the Purkinje cells showed shrinkage and pyknosis in some instances, and vacuolization and chromatolysis in others. Many Purkinje cells had completely disappeared. Normal boutons terminaux were present about the perikarya of both the pyknotic and vacuolated cells in Bodian preparations.

Dog No. 13, a normal adult male, subjected to arrest of the circulation of the brain for 8 minutes, was sacrificed at the end of 2½ months. Hematoxylin and eosin, and thionin preparations showed that almost all of the Purkinje cells had disappeared (Fig. 1). In many folia, no Purkinje cells could be found. The few Purkinje cells remaining were obviously pathological. Material stained by the Bodian method revealed baskets of nerve fibers surrounding the absent Purkinje cells (Fig. 3) and within these baskets small-looped synaptic terminals of normal morphology were observed. Normal boutons terminaux were also demonstrable on the severely damaged Purkinje cells which were still present.

Dog No. 18,* an adult female in the last week of pregnancy, was subjected to 2 minutes of arrest of the circulation of the brain and was sacrificed at the end of 2½ months. Examination of the thionin preparations revealed that while relatively few Purkinje cells had disappeared, almost all of the cells showed pathological changes such as chromatolysis, shrinkage and pyknosis, and vacuolization. Normal boutons terminaux were seen on the damaged Purkinje cells in preparations stained by the Cajal method. With the phosphotungstic acid-hematoxylin stain, marked proliferation of astrocytes around the pathological Purkinje cells was evident.

Dog No. 6,* an actively lactating adult female subjected to

* Pregnancy, lactation and puberty rendered animals more susceptible to arrest of the brain circulation.¹²

arrest of the circulation of the brain for 4 minutes, remained in coma and survived for 6 days. Thionin-stained sections showed a moderate disappearance of Purkinje cells but the cells remaining all showed shrinkage, pyknosis, eccentricity of the nucleus with marked folding of the nuclear membrane, severe chromatolysis or vacuolization. In Cajal preparations, normal boutons terminaux were found in the tangled masses of axons which remained at the site of completely disintegrated Purkinje cells. Normal synaptic terminals were also observed on the pathological Purkinje cells.

*Dog No. 7,** a female in her first estrus subjected to 6 minutes of arrest of the circulation of the brain, remained in coma and survived for 9 days. Practically all of the Purkinje cells had disappeared from the cerebellar cortex. On pathological fragments of Purkinje cells which remained as well as in the baskets of fibers at the site of the absent Purkinje cells, morphologically normal boutons terminaux were found.

*Dog No. 24,** an adult female in early pregnancy subjected to arrest of the circulation of the brain for 7 minutes, remained in coma and survived for 11 days. The sections stained by Cajal's method revealed almost complete disappearance of the Purkinje cells. Normal boutons terminaux remained in the baskets of fibers surrounding the site of the completely disintegrated Purkinje cells (Fig. 4).

DISCUSSION

The problem of the relative resistance to lack of oxygen of different portions of the nerve cell is a difficult one and no conclusive evidence is yet available. While the hypothesis that the synaptic ending is the portion of the neuron most susceptible to anoxia has been widely accepted, the evidence on which this hypothesis is based is meager and inconclusive. Wolff³ stated that vascularity varies quantitatively with the number of synaptic structures and does not vary with differences in the number or mass of nerve-cell bodies. On the basis of studies of vascularity of various portions of the nervous system, he suggested a higher metabolic rate and greater sensitivity to anoxia at the synapses. Similar data on vas-

* Pregnancy, lactation and puberty rendered animals more susceptible to arrest of the brain circulation.¹²

MULTIPLE SKIN TUMORS IN MICE FOLLOWING A SINGLE PAINTING WITH 9:10 DIMETHYL-1:2 BENZANTHRACENE *

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The chief problem with which most investigators of experimental "tar" cancer have been concerned is whether carcinogenic agents induce cancer because of their constitutional effects or whether the reaction produced is merely a local manifestation. This is particularly difficult to determine. As Seelig and Cooper¹ pointed out, most reasoning has been indirect and as yet there is no satisfactory solution of the problem.

During the course of an experiment testing the susceptibility of different inbred lines of mice to painting with the carcinogen 9:10 dimethyl-1:2 benzantracene,‡ some interesting data were obtained concerning the reaction of the C57 brown strain of mice. Previously, Mider and Morton² had shown this strain to be especially sensitive to paintings with methylcholanthrene.

The C57 brown strain of mice has been maintained at this laboratory for more than 40 generations of brother-sister matings. Epithelial tumors are rare. Mammary tumors appear in approximately 5 per cent of the breeding females while spontaneous papillomata and carcinomata, other than mammary, have but rarely been observed.

Thirty mice 4 weeks of age were painted twice a week mid-dorsally from the occiput to the near lumbar region with a 0.3 per cent solution of 9:10 dimethyl-1:2 benzantracene in thiophene-free benzene. This was applied with a No. 6 camel's hair brush. Paintings were continued for 12 weeks. More than half of these mice developed multiple persisting papillomata or carcinomata. All but two of these growths appeared within the painted area. The majority of mice surviving the toxic effect of the carcinogen developed a leukemoid condition involving only the liver and spleen.

Ten mice were given a single application of the carcinogen in the same region. After the solution had completely evaporated,

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† Finney-Howell Foundation Fellow.

‡ Supplied through the courtesy of W. E. Bachmann, University of Michigan.

cularity have been reported by Lorente de Nò¹³ and Campbell.¹⁴

Data concerning the relative vascularity of different parts of the nervous system cannot serve as a sound basis for the determination of the relative metabolic rate and resistance to anoxia of different parts of the neuron. Areas rich in synapses are not always highly vascular.⁴ Furthermore, the quantitative correlation between metabolic rate and resistance to anoxia on the one hand and vascularity on the other hand is generally poor.⁶ A striking example of this lack of correlation is provided by the supra-optic nucleus in the hypothalamus. This nucleus, which has the greatest vascularity of all nerve centers in the brain,¹⁵ is nevertheless among the most resistant to destruction by arrest of the circulation of the brain. In a dog in our series subjected to arrest of the circulation of the brain for 19 minutes and surviving for 9 days, the supra-optic nucleus appeared histologically normal despite disintegration and disappearance of the nerve cells of the cerebral cortex, Purkinje layer of the cerebellum, corpus striatum, thalamus and many nuclei of the brain stem as far caudally as the medulla.

A direct study by Bronk⁵ of loss of function of the stellate ganglion as a result of anoxia revealed that the perikaryon was the first structure to lose its irritability. In this type of experiment, it was impossible to determine whether function was maintained in the synapse after suppression of activity in the ganglion cell. While it is possible either that the synapse may continue to function much longer than the cell body or that the two structures may lose their irritability at about the same time, it is certain that the synapse is not *more* susceptible to anoxia than the perikaryon. In an attempt to determine the rate of oxidative metabolism of different portions of the neuron by the reduction of ferric chloride, Gerard⁶ found that the apparent metabolism was much higher in the perikaryon than in neuropil in the brain. While the technic employed is admittedly crude, the results are suggestive.

The available information on neurons of the central nervous system, the axons of which terminate peripherally, also suggests a greater resistance to anoxia of the terminals. The neurons of the lateral horn of the spinal cord undoubtedly cease functioning much earlier, due to anoxia, than do their terminal axons which form the synapses in the stellate ganglion. Similarly, the anterior horn

these mice were placed 2 or 3 in a cage and given the same food—purina fox chow—as the first group. Epilation occurred within 1 week, followed by a growth of white hair in the epilated region. There was no ulceration of the skin.

A papilloma appeared on the right foreleg of ♂ B₁, 169 days after painting. This was found to be carcinomatous at approximately 238 days. The second growth appeared on the right foreleg of ♂ B₂₃, 199 days following painting. This proved to be carcinomatous at 224 days. Following these there appeared in all, twenty-five papillomata on the 10 mice given single paintings (Table I). There were eight growths on ♂ B₁: four of these be-

TABLE I
Multiple Skin Tumors Following a Single Painting with 9:10 Dimethyl-1:2 Benzanthraccene

Mouse No.	Papillomata		Carcinomata		Position
	No.	Appearance (days)	No.	Appearance (days)	
♂ B ₁	8	169 249 339; regressed 314; regressed 279 274; persisted 327; regressed 281	4	238 280 339 329	Dorsal—OPA* Dorsal—OPA Dorsal—OPA Within painted area Within painted area Dorsal—OPA Ventral Ventral
♂ B ₂	4	224 240; persisted 254 314; persisted	2	254 299	Dorsal—OPA Dorsal—OPA Within painted area Dorsal—OPA
♂ B ₃	2	270 240	2	300 272	Dorsal—OPA Dorsal—OPA
♀ B ₈	1	377	1	421	Ventral
♀ B ₉	3	279 335; regressed 305; regressed	1	319	Ventral Ventral Dorsal—OPA
♂ B ₂₂	2	240; persisted 330; persisted	0		Dorsal—OPA Ventral
♂ B ₂₃	1	199	1	224	Dorsal—OPA
♂ B ₂₄	2	240 274; regressed	1	347†	Ventral Dorsal—OPA
♀ B ₂₉	1	304; regressed	0		Ventral
♀ B ₃₀	1	283	1	328‡	Dorsal—OPA

* OPA = outside painted area.

† Transplanted subcutaneously to 8 C57 brown mice. Positive malignancy.

‡ Transplanted subcutaneously to 7 C57 brown mice. Positive malignancy.

cells are more susceptible to anoxia than are their terminal axons forming the motor end-plates. These perikarya not only lose function more readily but are also much more susceptible to destruction by anoxia than are the terminals. While the myoneural junction is not a synapse, its functional similarity to the synapse is now well established.

The results of our own investigation demonstrate clearly that the Purkinje cells of the cerebellar cortex can be destroyed by arrest of blood flow and entirely removed without producing evident structural changes in the related synaptic terminals. This has been observed in material following various periods of complete arrest of the circulation of the brain and after varying intervals of survival. The period of survival of our animals would seem to be sufficiently long to bring out pathological changes in, or disappearance of, the boutons terminaux, should such occur following this procedure. Hoff ¹⁰ described marked distinctive alterations in the boutons terminaux within 4 days and their disappearance within 6 to 7 days after section of the axon.

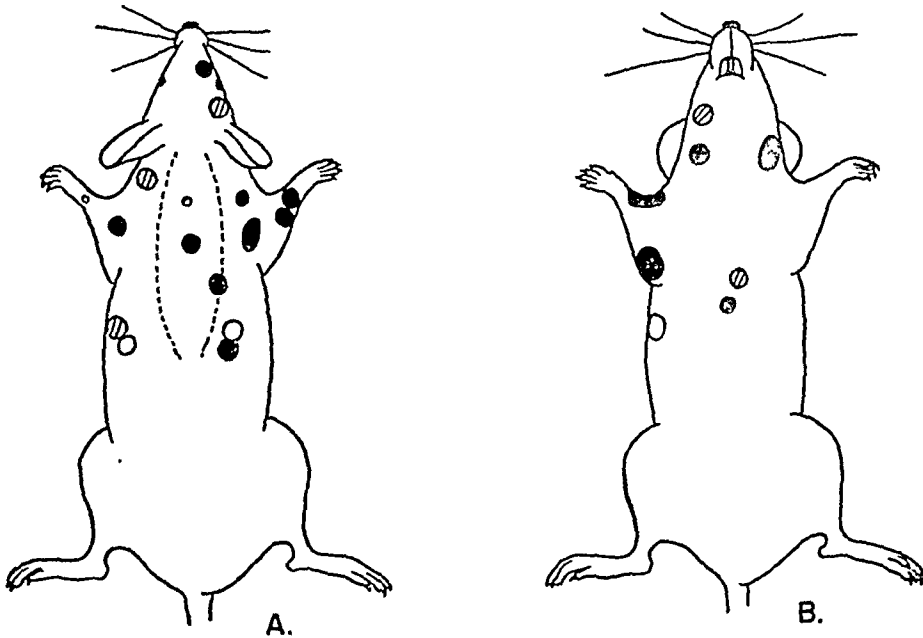
While these results indicate that the synaptic terminals on the Purkinje cell are more resistant to damage by arrest of circulation than is the perikaryon, little information is available concerning the quantitative differences in the resistance of the two structures. Moreover, while the correlation between different indices of resistance to anoxia, such as loss of function and permanent morphological damage, is usually good,⁶ direct evidence on the relative rapidity of loss of function of synapses and perikarya in the brain is lacking. In general, there is also close correlation between resistance to anoxia and metabolic rate.⁶ One cannot overlook the possibility that different synapses in the brain may show great differences in resistance to anoxia in the same way that different neurons show such differences.

The results of this investigation are also of interest, incidentally, in relation to the much debated problem of contiguity or continuity at synapses. Many authors, especially Cajal,¹⁷ championed the view that there is no continuity across synapses. This has been questioned by a number of investigators, a recent supporter of structural continuity across the synapse having been Tiegs.¹⁸ The fact that the synaptic terminals on the Purkinje cell retain their normal structure after disintegration and disap-

came carcinomata, three regressed, while one persisted until necropsy as a papilloma.

Thirteen of the papillomata became transformed into carcinomata, seven regressed completely and five persisted as such without much apparent change. Malignancy was detected by a decided change in the rate of growth at the base of the lesion, followed usually by ulceration. In some cases diagnosis was confirmed by biopsy. Histological study was made of all lesions.

Twenty-two of the tumors appeared outside of the painted area (Text-Fig. 1). These were confined mostly to the forelegs and head region. Eight growths appeared on the near-ventral or ventral region. It is interesting to note that none of the tumors



TEXT-FIGURE 1. Distribution of skin tumors in C_{57} brown mice given a single application of 0.3 per cent 9:10 dimethyl-1:2 benzanthracene in benzene. A, dorsal; B, ventral. Solid areas indicate carcinomata, hatched areas indicate papillomata persisting as such, unmarked areas indicate papillomata which regressed. Dorsal painted region shown by dotted lines.

appeared behind the base of the painted area (lumbar region), whereas numerous growths appeared in front of the tip of the painted area (occiput region).

Two of the malignant growths were transplanted to 1-month-old mice of the C_{57} brown strain. Both proved to be slowly growing carcinomata which invaded muscle. One of these growths

pearance of the neuron may be taken as evidence against structural continuity across the synapse.

The hypothesis that the synaptic ending is the most sensitive to anoxia of the various portions of the neuron has been widely accepted despite the lack of conclusive confirmatory evidence. While accurate data on the relative metabolic rates and resistance to anoxia of different parts of the nerve cell are not yet available, the evidence thus far would suggest caution in accepting the traditional view.

CONCLUSIONS

1. Complete temporary arrest of the circulation of the brain results in disintegration and removal of the Purkinje cells of the cerebellar cortex, but does not alter the morphological character of the associated boutons terminaux.

2. The relation of this observation to the problem of the relative resistance to anoxia of different portions of the neuron is discussed.

NOTE: We should like to express our appreciation to A. T. Rasmussen for his kind advice and assistance.

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in ♂ B23 had much the appearance of adenocarcinoma of a mammary gland.

In 1934, Reinhard and Thibaudeau ³ called attention to a peculiar and unusual development of multiple tumors of the skin in an inbred strain of mice painted with tar for a period of from 8 to 12 weeks. The tumors appeared both dorsally and ventrally. These growths, mostly sebaceous adenomata in the final stage, were not characteristic, however, of ordinary tar tumors. In a later report, Reinhard and Candee ⁴ suggested that ingestion of the tar material from the backs of mice or from the cages may account for this unusual phenomenon. This seems a very remote possibility in our experiment since the mice were given only one application. Furthermore, the growths produced were identical with those resulting from continuous paintings and were restricted to the anterior part of the body.

It has been found by the British group ⁵ that epitheliomata produced by multiple paintings with a 0.06 per cent solution of the 5:9:10 trimethyl-1:2 benzanthracene compound appeared at the edge of the painted area and not at its center, suggesting that the optimal conditions for tumor production are in the regions of considerable dilution. Again, such an explanation would fail to account for those growths appearing on the forelegs and on the near-ventral and ventral surfaces.

Since mice of this strain receiving multiple paintings of 9:10 dimethyl-1:2 benzanthracene developed lesions almost entirely within the painted area, it seems very unlikely that scratching or accidental brushing would account for the unusual distribution of growths in singly painted mice. In fact, the position of some of the abnormal growths is such that scratching could not account for their appearance.

Certain definite conclusions can be drawn. A very small amount of carcinogen is required to initiate abnormal growth of the epithelial cells in mice of the C57 brown strain. Once a benign growth is begun, no further external application of carcinogen is required to produce malignancy. The solution to the question of whether these induced tumors result from a general constitutional effect must await further experimentation.

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DESCRIPTION OF PLATE

PLATE 133

- FIG. 1. Photomicrograph of the cerebellar cortex of dog No. 13, stained with hematoxylin and eosin. This illustrates the disappearance of Purkinje cells resulting from 8 minutes of arrest of the circulation of the brain. $\times 85$.
- FIG. 2. Photomicrograph of the cerebellar cortex of a normal dog, stained by the Cajal block silver method. This illustrates the normal boutons terminaux on the Purkinje cell. $\times 860$.
- FIG. 3. Photomicrograph of the cerebellar cortex of dog No. 13, stained by the Bodian silver method. The animal was subjected to 8 minutes of arrest of the circulation of the brain. The Purkinje cells have disappeared but the baskets of nerve fibers surrounding them persist. $\times 380$.
- FIG. 4. Photomicrograph of the cerebellar cortex of dog No. 24, stained by the Cajal block silver method. The animal was subjected to 7 minutes of arrest of the circulation of the brain and survived for 11 days. The Purkinje cell has completely disintegrated and disappeared. Normal boutons terminaux persist in the basket of nerve fibers surrounding the site of the disintegrated Purkinje cell. $\times 940$.

this route died in 14 hours, 2 days, 6 days, and 15 days after inoculation. Severe lesions were found in all.

REPRESENTATIVE PROTOCOLS

Rabbit No. 1.

11/16/38. 11:00 a.m.: blood sugar, 102 mg. per 100 cc.; t. 103°; insulin, 2 units.

1:30 p.m.: blood sugar, 38 mg.; 0.4 cc. virus suspension injected.

3:30 p.m.: convulsions; glucose administered.

4:00 p.m.: rabbit fully recovered.

9:00 p.m.: blood sugar 226 mg.; t. 104.5°; all extremities were weak; eyelids drooped; respirations rapid.

11/17/38. 2:30 p.m.: t. 103.4°; refused food; all extremities paretic; condition worse.

11/18/38. 10:00 a.m.: t. 102.4°; extremities flaccid.

2:30 p.m.: died.

Autopsy: Cut surface of medulla and cord at various levels showed gray matter to be hemorrhagic and soft. Microscopic examination at various levels revealed severe necrosis of the anterior horn cells and in some sections the neurons had disappeared (Fig. 1).

Rabbit No. 13.

3/11/39. 9:00 a.m.: blood sugar, 120 mg. per 100 cc.; t. 103°.

9:15 a.m.: insulin, 1 unit.

10:10 a.m.: 0.4 cc. virus suspension injected.

10:15 a.m.: t. 102°; blood sugar, 100 mg.

11:45 a.m.: blood sugar, 66 mg.

12:30 p.m.: t. 102.8°.

1:30 p.m.: blood sugar, 40 mg.; convulsions; glucose administered.

2:30 p.m.: t. 100°.

4:00 p.m.: looked ill; limp; eyelids drooped; coarse tremors of hind legs.

11:00 p.m.: died.

Autopsy: Cord grossly hyperemic and edematous. Microscopic examination showed widespread moderate to severe necrosis of anterior horn cells (Fig. 2).

B. INTRANASAL INSTILLATION. Three rabbits were inoculated by this route. One died on the sixth day and the other 2 were killed on the sixth and twelfth days. Lesions were found in all, but were most severe in the rabbit that died spontaneously.

REPRESENTATIVE PROTOCOL

Rabbit No. 18.

2/18/39. 5:45 p.m.: t. 102.7°; blood sugar, 105 mg. per 100 cc.; insulin, 0.8 unit.

6:40 p.m.: t. 100.6°; blood sugar, 60 mg.
 7:00 p.m.: 1 cc. virus suspension instilled into each nostril.
 8:05 p.m.: t. 100.5°.
 8:30 p.m.: blood sugar, 80 mg.
 8:50 p.m.: 1 cc. virus suspension instilled into each nostril.
 10:10 p.m.: t. 101.6°.
 11:15 p.m.: 1 cc. virus suspension instilled into each nostril.

2/19/39. 12:20 a.m. t. 102.2°.

2/20/39. t. 102.0°.

2/21/39. t. 102.3°.

2/22/39. t. 102.0°.

Autopsy: Surface of brain and cord hemorrhagic and edematous; cut surface of cord, especially in the thoracic region, showed gray matter to be prominent and hyperemic. Microscopic examination revealed scattered neuronal necrosis most marked in the thoracic levels (Fig. 3).

II. Rabbits Injected with Suspensions of Rabbit Cord Virus.

A. INTRACEREBRAL INJECTION. Two rabbits inoculated by this route died spontaneously on the third and fourth days. Both showed severe lesions.

REPRESENTATIVE PROTOCOL

Rabbit No. 16.

3/27/39. 7:40 p.m.: t. 103°; blood sugar, 110 mg. per 100 cc.; insulin, 1.6 unit.

8:30 p.m.: 0.4 cc. cord suspension from rabbit No. 13.

9:15 p.m.: t. 104.2°.

10:25 p.m.: hypoglycemic symptoms; blood sugar, 45 mg.; glucose administered.

3/28/39. 2:00 p.m.: t. 102°; looked ill; eyelids drooped; no paralysis.

3/29/39. 2:00 p.m.: t. 101°; condition worse; paresis of extensor muscles of head and neck.

3/30/39. Died.

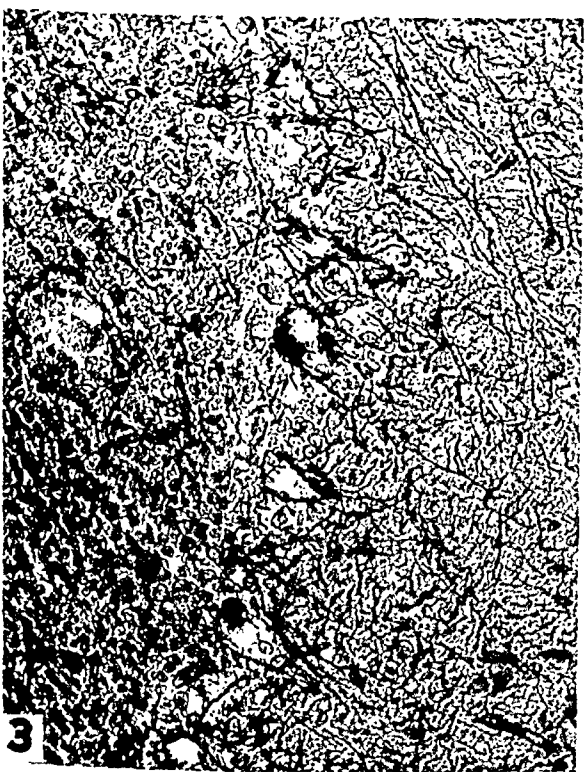
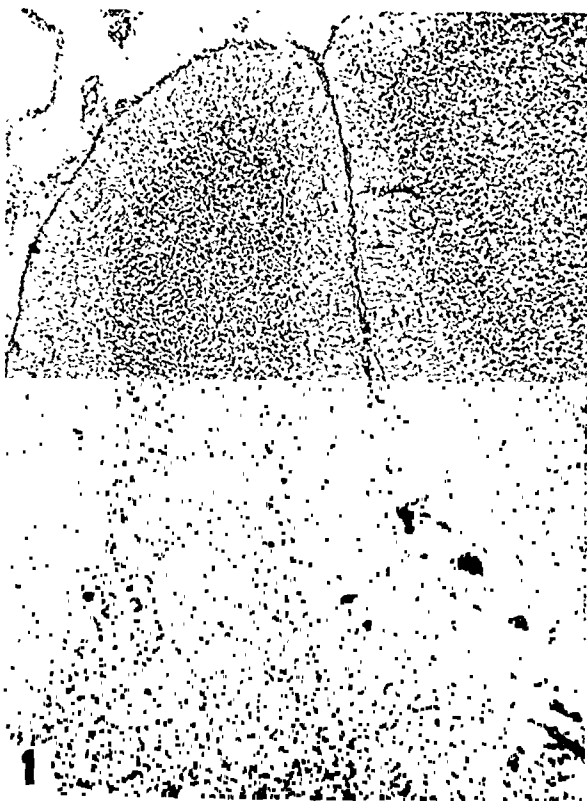
Autopsy: Cut surface of cord showed gray matter to be prominent and hemorrhagic. Microscopic examination revealed severe neuronal necrosis at various levels.

B. INTRANASAL INSTILLATION. Two rabbits were inoculated by this route. One died on the third day and the other was killed on the fifth day. Both showed lesions.

REPRESENTATIVE PROTOCOL

Rabbit No. 17.

4/1/39. 7:00 p.m.: t. 102.2°; blood sugar, 100 mg. per 100 cc.; insulin, 1.5 unit.



Kabat and Schadewald

Relative Susceptibility to Anoxia

Bell,¹⁸ Warwick,¹⁹ and Matsuoka.²⁵ Frequently, however, the ratio as stated is misleading due to the fact that allowance is not made for an undue proportion of one sex occurring in an age period of high incidence.

Appendiceal Oxyuriasis and Appendicitis

Location of Worms. Gordon²¹ and Warwick's¹⁰ studies indicate that appendiceal oxyuriasis is gradually increasing in fre-

TABLE V
Incidence of Appendiceal Oxyuriasis as Related to Sex

Age groups (years)	Author's series	Gordon's series*		
	Ratio of percentage of positive cases, male to female	Percentage positive, male	Percentage positive female	Ratio of percentage of positive cases, male to female
2-6	1 - 1.33	9.99	8.11	1 - 0.81
7-11	1 - 1.48	4.43	5.03	1 - 1.14
12-16	1 - 2.41	1.61	3.00	1 - 1.86
17-21	1 - 2.26	0.38	0.87	1 - 2.29
22-26	1 - 2.19	0.27	1.01	1 - 3.74
27-31	1 - 1.38	0.96	0.98	1 - 1.02
32-36	1 - 2.15	0.25	0.55	1 - 2.20
37-41	1 - 1.56	0.34	1.20	1 - 3.53

* Percentages and ratios computed from Gordon's⁸ Table I.

quency. This increases the importance of the possible causal relationship of oxyuriasis to appendicitis. Much of the evidence that has been submitted is of a circumstantial nature and cannot be relied upon for definite conclusions. The direct evidence upon which a justifiable conclusion should be based is the demonstration of inflammatory lesions in the wall of the appendix due to parasitic invasion. Oxyurids have been found by many investigators at varying depths in the wall but there is a difference of opinion as to whether the invasion took place preoperatively or postoperatively. Of the 184 appendices in our series that contained oxyurids, 79 were found positive by histological examination. Only one of these showed invasion of the wall. In this case the worm was present in the mucosa just within the margin of a lymphoid follicle, lying in a smooth-walled space. The marginating lymphoid tissue showed prominent pressure condensation of the lymphocytes, but there was no evidence of any inflammatory reaction and hemorrhage was not present. In 65 appendices the worms were present only in the lumen and were not in contact with the mucosa. In 13, one or more oxyurids were in contact

with the mucosa; of these, 7 showed shallow crescent-shaped depressions with from slight to marked atrophy of the surface epithelium; rarely nuclei showed karyorrhexis. Hemorrhage was not seen in any of these, and there was no inflammatory reaction in the underlying lamina propria. Gordon,²¹ in 311 positive cases, found 33 in which oxyurids had penetrated to various depths in the wall. In none of these was there any inflammatory reaction in the surrounding tissue. This fact, together with other evidence, led him to the conclusion that invasion had taken place post-operatively. Cecil and Bulkley,⁹ Botsford, Hudson and Chamberlain²⁰ and Askanazy²⁶ also noted the absence of inflammation around the invading worm. The latter author, however, stated that in a rare case the invading oxyurid dies in the tissue and initiates a proliferative pseudotuberculous reaction. From the photomicrographs he submits to illustrate this process it is impossible to identify the foreign material as an oxyurid. Harris and Browne¹⁵ also found oxyurids in the appendiceal wall, but in their cases associated inflammatory changes were present.

This laboratory repeatedly emphasizes the necessity of immediate fixation of specimens that are to be submitted for histologic examination. This fact is interesting in view of our single example of invasion by an oxyurid. Bearing on this subject is the fact that Beck¹⁶ and Warwick¹⁹ removed the appendiceal contents immediately after operation. On histologic examination they did not find a single appendix showing worms in the wall.

Relationship of Incidence to "Pathologic Type." The evidence most frequently presented by those who deny any relationship between oxyuriasis and appendicitis is the common occurrence of these worms in normal appendices and their low incidence in acute appendicitis. Table VI lists both positive and negative cases by pathologic diagnosis. The incidence of infestation in the normal appendices and in those showing chronic appendicitis is about equal, being 10.9 per cent for the former and 10.2 per cent for the latter. All appendices showing acute or subacute inflammation, with the exception of the acute catarrhal group, had a much lower rate of infestation. The incidence for the acute catarrhal group is 8.1 per cent. Most investigators agree that oxyurids are relatively infrequent in suppurating appendices; however, there is considerable divergence of opinion as to their frequency in

APPENDICEAL OXYURIASIS *

ITS INCIDENCE AND RELATIONSHIP TO APPENDICITIS

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Studies on the incidence of appendiceal oxyuriasis (enterobiasis) and the possible causal relationship of this organism to appendicitis have appeared with increasing frequency since 1900. Stimulation of interest in this subject is generally credited to the work of Still¹ and Metchnikoff² who emphasized the probable relationship of appendicitis and intestinal helminthiasis.

The incidence of appendiceal oxyuriasis, as reported by different investigators, varies from 0 to 100 per cent. This marked variation may occur from the same country or in the report of one author on two or more series of cases. Crile³ did not find a single case in the material from 1,000 appendectomies whereas Fischer⁴ found 46 positive cases in 110 and Nicolaus⁵ found all of 5 surgically removed appendices to be positive. Aschoff⁶ in one series found oxyurids in 14 of 78 specimens but in another series⁷ of 1,000 appendices oxyurids were found in but 2.

The life history of *Enterobius (Oxyuris) vermicularis* and the mechanism of human infestation make it reasonable to expect that the incidence of oxyuriasis should vary when reports are based on different races or race groups. However, much of the confusion results from the small number of cases in the various series reported, failure to consider the age and sex factors, and the varying methods used to determine the presence or absence of the worms. Gordon's⁸ report based on a large number of cases is one of the few that records adequate pertinent data concerning age and sex. From his rather complete review of the literature, he concluded that "appendiceal oxyuriasis occurs the world over though with varying frequency." Since a detailed listing of the varying incidence figures for Europe would serve no useful purpose, only the averages as compiled by Gordon⁸ will be given. For Great Britain the reported incidence is 18.5 per cent; for France,

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other pathologic types. This is probably due to the non-standardization of criteria for the diagnosis of appendicitis. There have been a few reports showing infestation to be higher in inflamed than in normal appendices. These, however, are in the minority. Innes and Campbell²⁷ stated that in no case did they find worm infestation in a "healthy appendix."

Activity of Inflammation and Incidence. In this laboratory, chronic appendicitis, from a pathologic standpoint, is divided into

TABLE VI
Pathological Diagnoses and Incidence of Oxyuriasis

	No. appendices	No. with oxyurids	Percentage positive
Normal	55	6	10.91
Chronic	1378	141	10.23
Chronic and acute	225	12	5.33
Chronic and subacute	159	7	4.40
Acute	323	8	2.48
Acute catarrhal	37	3	8.11
Subacute	122	7	5.74
Tuberculosis, carcinoid, appendiceal abscess	18	0

two groups: inactive and active. Appendices in the former group show from slight to marked increase in fibrous tissue in the submucosa and serosa, and occasionally scarring of the muscularis. These findings are considered evidence of past damage. The minimum requirement for placing an appendix in the active group is the finding of slight to marked phagocytic activity of the follicular reticulum cells. There is frequently other evidence of chronic activity, such as variable numbers of lymphocytes in the muscularis and serosa. Of the 1,378 appendices that were diagnosed as chronic appendicitis, 479, with 7.1 per cent positive for oxyurids, showed no phagocytic activity of the follicular reticulum cells. The remaining 899 showed slight, moderate or marked phagocytic activity. There were 8.9 per cent positive for oxyurids in the 463 appendices with slight activity, 15.5 per cent positive in the 369 with moderate activity and 13.4 per cent in the 67 appendices showing marked phagocytic activity of the follicular reticulum cells. These figures indicate that in the chronic appendicitis group, appendiceal oxyuriasis occurs more frequently in cases showing an active pathologic process. It should be emphasized, however, that a considerable number of positive appendices

11.9 per cent; and for continental Europe, 3.7 per cent for surgical, and 16.5 per cent for autopsy specimens.

The American literature, although less voluminous than the European, also shows this marked variation in incidence. Cecil and Bulkley⁹ found 20 (13.5 per cent) positive cases in 148 appendices from children. Crile³ did not record a single case in 1,000 appendices. Deaver¹⁰ and Hanley¹¹ each found 1 (0.2 per cent) appendix containing oxyurids in series of 500 cases. Erdmann¹² found 4 appendices infested with oxyurids; however, it is not clear from his report whether they represent the positives in the entire series of 201, or occurred in the 22 cases of appendicitis among children. Ney¹³ found 3 (3 per cent) positive in 100 appendices; Garlough,¹⁴ 4 (4.2 per cent) in 96; Harris and Browne,¹⁵ 22 (18.2 per cent) in 121; Beck,¹⁶ 35 (2.04 per cent) in 1,718; Goodale,¹⁷ 101 (6.2 per cent) in 1,639; Bell,¹⁸ 15 (10.9 per cent) in 138; Warwick,¹⁹ 45 (1.9 per cent) in 2,344; and Botsford, Hudson and Chamberlain,²⁰ 71 (5.3 per cent) in 1,343. Gordon⁸ in 1931, reporting on 20,969 appendices, found 221 (1.05 per cent) which contained oxyurids on histological examination. In 1933,²¹ reporting on an additional 5,082 appendices, he records 90 positive cases, giving an incidence of 1.19 per cent for the entire series of 26,051. Gordon's report, in contrast to most of those referred to above, gives age and sex grouping for both the positive and negative appendices. Comparison of percentages of incidence is of value only if allowance has been made for age and sex.

MATERIAL AND METHODS

From May 1, 1936 to January 11, 1939, 2,616 surgically removed appendices were received for diagnosis. Of these, the lumen was completely obliterated in 91. Ninety-two had been longitudinally opened by the surgeon. Twenty-four were improperly fixed and in 92 cases age and sex data were lacking. These 299 appendices were excluded, leaving a total of 2,317 for use in this study.

The specimens were received from the United States Public Health Service hospitals, located mainly along the east and west coast, and from hospitals of the United States Indian Service. At the former sources, the patients were mainly American seamen or United States war veterans, and at the latter they were full or mixed-breed American Indians living in midwestern and south-

showed no active lesions; also, when chronic appendices were compared with normals (Table VI), the incidence of oxyuriasis was not significantly different. These facts emphasize the fallacy of relying too much on circumstantial evidence.

In none of the positive cases showing inflammation was there any evidence to suggest that the worms were etiologically related to the appendicitis.

Oxyurids and Fibrosis. Rheindorf²⁸ offered the theory that oxyurids in the appendix cause an increase in connective tissue deposition. This relationship of oxyurids to sclerosis has been denied by Aschoff.⁷ In this connection our data are of interest. There were 225 appendices which showed absence of mucosa with fibrous obliteration of the lumen. Of these, 55 showed obliteration of the distal half, and in 170 only the distal third was involved. In this group, oxyurids were present in 6 appendices (2.67 per cent). Since most of the 225 cases were in the chronic appendicitis group (showing 10.2 per cent infestation), the low incidence in this "obliterative" group is ample evidence against the Rheindorf theory.

Local Eosinophilia. There have been a number of authors who have commented on the relation of local eosinophilia and appendiceal oxyuriasis. Fischer,⁴ in his study, found that appendices containing the greatest number of oxyurids frequently showed the most marked local eosinophilia; however, he found some with marked eosinophilia and no parasites. Beck¹⁶ stated that in her series local eosinophilia was not significantly increased in the positive cases. Gordon,²¹ in a controlled study, found local eosinophilia as often in the negative appendices as in those containing oxyurids. Eastwood²⁹ came to the same conclusion. His report is based on 73 inflamed appendices and 50 normal ones. The inflamed group included those showing varying degrees of activity; hence local eosinophilia was markedly affected by this factor, which would mask or accentuate any possible relationship between the presence of oxyurids and local eosinophilia.

Since the degree of appendiceal eosinophilia varies with the degree of inflammation, only appendices showing chronic inflammation were used in the comparison shown in Table VII. The degree of eosinophilia was graded by one individual. It was considered possible that finding oxyurids in the section might have

western parts of the United States or in Alaska (Eskimos and Aleutians).

The appendices were fixed in a 4 per cent aqueous solution of formaldehyde for 24 hours or longer, and representative blocks were taken from the proximal, mesial and distal thirds for paraffin embedding. The contents of the remainder of each appendix, in 1,319 cases, were expressed into a small vial containing a 4 per cent aqueous solution of formaldehyde and examined later under a dissecting microscope for the presence of oxyurids. In many cases contents could not be expressed. In some others the amount obtainable by this method was considered inadequate for a satisfactory examination.

Duplicate sections were routinely made of the embedded material and one each stained by the Romanowsky and van Gieson methods. When only a few worms were present they were found in only one level. This fact emphasized the importance of examining the contents of the unembedded portion of each appendix. By this procedure it was possible to determine the number of positive cases that are missed when histological examination alone is relied upon.

More than half of the histopathologic diagnoses were made by me. Although the remainder were made by three other members of the divisional staff, the criteria for diagnosis had been standardized so that no difficulty was experienced in placing a given appendix under its proper diagnostic heading.

In order to make certain comparisons, to be discussed later, the sections of appendices were classified in the following three groups: "contracted," "open and empty," and "open and full." An appendix with a lumen less than 2 mm. in diameter was placed in the first group. If the lumen was greater than 2 mm. ("open"), it was placed in the second or third group, depending on the presence or absence (lost during preparation) of fecal contents.

RESULTS

The Effect of the Method of Examination on the Percentage of Incidence

The incidence of appendiceal oxyuriasis, as shown by different reports, has been based on gross inspection, on examination of stained sections or on microscopic examination of the entire ap-

TABLE VII
Appendiceal Oxyuriasis and Local Eosinophilia

Increase in eosinophils	Negative for oxyurids		Positive for oxyurids	
	By histologic examination	By examination of appendiceal contents	By histologic examination	By examination of appendiceal contents
None	440 (82.2%)	617 (82.2%)	44 (65.7%)	62 (77.5%)
Slight	75 (14.0%)	93 (12.4%)	16 (23.9%)	15 (18.8%)
Moderate	18 (3.4%)	40 (5.3%)	6 (8.95%)	2 (2.5%)
Marked	2 (0.37%)	1 (0.13%)	1 (1.5%)	1 (1.25%)
		17.8%	34.3%	22.5%

some psychological effect on the grading, hence a comparison was made between the appendices which were positive in sections and those in which oxyurids were found only by examination of the removed appendiceal contents. The results showed this check on grading to be worth while. Of the appendices positive in section, 34.3 per cent showed local eosinophilia whereas only 22.5 per cent of those positive by the second method of examination showed such an increase.

The controls (negative cases) were also divided into two groups, to determine what effect "chance" would have on the recognition of local eosinophilia. There were 751 appendices which were negative by examination of the removed contents. Of this group, 17.8 per cent showed an increase in eosinophils. Of the remaining 535 negative cases, examined only in section, 17.8 per cent showed local eosinophilia. From this one must conclude that the higher percentage of appendices with local eosinophilia which occurred in one of the positive groups was due to the psychological effect of the observed presence of the worm, when the grading was done.

Local eosinophilia was only 4.7 per cent more frequent in the positive than in negative appendices. This difference cannot be accepted as statistically significant, especially since the difference between the two positive groups (11.8 per cent) was more than twice this figure.

SUMMARY AND CONCLUSIONS

1. In a series of 2,317 surgically removed appendices, oxyurids were found

pendiceal contents. Only by the latter method can a correct rate be calculated. By histological examination, Gordon²¹ found oxyurids in 1.19 per cent and Warwick¹⁹ in 1.9 per cent of their cases; and by microscopic examination of the removed appendiceal contents, Goodale¹⁷ found 6.1 per cent and Beck¹⁶ 2.03 per cent positive. These percentages indicate only the relative effectiveness of the two methods since the effect of age and sex on incidence is not shown in these figures. Bell's¹⁸ reported incidence of 10.9 per cent is based on examination of contents, but he does not state whether or not microscopic examination was done.

In this study oxyurids were found in 133 (10.1 per cent) of 1,319 specimens from which appendiceal contents were expressed. Of this number, 28 were positive also in section, leaving 105 (57 per cent of the 184 total positives), which would have been missed if histological examination alone had been relied upon. This would have reduced the total incidence, for the series of 2,317 cases, from 7.9 per cent to 3.41 per cent.

In routine paraffin embedding, sectioning and staining, the contents are often lost from one or more of the three blocks. This is another factor productive of the lower incidence obtained by this method of examination. In this series there were 108 appendices, the lumina of which were "full" at all three levels. This group showed 5.56 per cent positive for oxyurids. There were 182 with two levels full and one empty, and 182 with one level full and two empty. These showed 5.49 per cent and 1.65 per cent positive, respectively.

Gordon's⁸ observation that when oxyurids were present in one block "the other two blocks were seldom completely devoid of worms" does not agree with the positive cases of this series. There were 34 positive appendices in which the lumina of all three blocks contained fecal material. Of this number only 4 (11.8 per cent) were positive in all three blocks; 7 (20.6 per cent) were positive in two blocks; and 23 (67.6 per cent) were positive in one level only. When all levels contained worms, the average number per block was 4.17; when two blocks were positive, the average for the two blocks was 2.15; and when only one level contained oxyurids, the average number was 1.48. As would be expected, these figures show that the probability of finding more

in 184 (7.94 per cent). Seventy-nine were positive in the sectioned material and in 105, oxyurids were found only by examination of the removed appendiceal contents.

2. Of the 34 appendices showing oxyurids in section and having fecal material in all three blocks, only one level was positive in 23 cases (67.65 per cent), two levels were positive in 7 (20.58 per cent) and all three levels were positive in 4 cases (11.76 per cent).

3. The incidence of appendiceal oxyuriasis was found to be 2.88 per cent for the white beneficiaries of the United States Public Health Service hospitals, 10.04 per cent for Indians of the United States, and 23.91 per cent for Eskimos and Aleutians.

4. The highest incidence occurred in the 7 to 11 year age group, being (for the entire series) 15 per cent for males and 22.22 per cent for females. The decrease in incidence was gradual until the 32 to 36 year period at which a second peak occurs, 7.44 per cent for males and 15.96 per cent for females. Appendiceal infestation is rare after 51 years of age.

5. Infestation is more common in appendices from females than from males. For the 2 to 46 year age period, the ratio is 1 male to 2.34 females.

6. Comparison of infested and control (negative) appendices showed that local eosinophilia is not significantly increased by infestation with oxyurids.

7. Oxyuriasis occurs as frequently in normal appendices as in appendices showing chronic inflammation and more frequently than in acutely inflamed appendices.

8. Of the 79 cases showing oxyurids in the lumen of the sectioned appendix, 13 showed the worm in contact with mucosa; 7 of these showed pressure atrophy of epithelium with formation of shallow crescent-shaped depressions. Rarely there was a little karyorrhectic necrosis, but no inflammation or hemorrhage was found.

9. One appendix showed an oxyurid in the deep part of the mucosa. The lack of inflammation, necrosis or hemorrhage shows that the invasion occurred postoperatively.

10. No case of "appendicitis oxyurica" occurred in this series. *Enterobius (Oxyuris) vermicularis* is not etiologically related to appendicitis.

than one block positive is roughly proportional to the total number of worms in the appendix. It also emphasizes the necessity of microscopic examination of the entire appendiceal contents in order to get an accurate incidence rate.

Race, Age and Sex

Generally, the figures for incidence for European countries are higher than those for the United States. This greater reported incidence is probably partly due to environmental conditions and race habits. In this study there is a prominent difference in incidence among the white population (seamen and veterans) of the United States, Indians of the United States, and Eskimos and Aleutians. In the former (Table II) the incidence is 2.88 per cent; in United States Indians, 10.04 per cent (Table III); and in Eskimos, 23.91 per cent (Table IV). These figures are not adjusted for age and sex. However, when these are corrected for sex and the age period of 12 to 41 years is used, the difference is equally prominent. For this age period, the incidence for white females is 3.64 per cent, for Indian females, 10.6 per cent, and for Alaskan females, 27.3 per cent. In Tables II, III and IV, both negative and positive cases are listed to show the effects of age, sex and race on incidence. In addition to the races mentioned, the total series (Table I) includes 1 Japanese, 1 Filipino, 10 Mexicans and 40 Negroes. The number of cases in the latter race is too small for reliable analysis; however, the finding of only 1 positive appendix in 40 (2.5 per cent) fits in with the observation of Cram, Jones, Reardon and Nolan²² that oxyuriasis occurs more frequently among the white population than among Negroes.

Since the effect of age and sex on incidence is comparable for the various races, the entire series is used in the discussion of these factors. It is generally agreed that appendiceal oxyuriasis is most frequent at the younger ages and relatively rare after the fifth decade. In this study the peak incidence occurs in the 7 to 11 year period (19.2 per cent) with a gradual fall to 4.7 per cent for the 27 to 31 year age group and then a sudden rise to 11.2 per cent for the next 5 year period. This secondary peak incidence is present in both white and Indian races, although occurring at a later period among the Indians. In Gordon's⁸ study, the highest incidence occurred in the 2 to 6 year period, whereas in our study

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TABLE I
Incidence of Appendiceal Oxyuriasis in 2,317 Appendices

Age groups	Males			Females			Both sexes						
	No.	With oxyurias	Per-centage positive	No.	With oxyurias	Per-centage positive	No.	Cases with oxyurias*					
								H+	H+ Z-	H- Z+	H+ Z+	Total positives	Per-centage positive
0-23 mo.	1	0	8.33	9	1	11.11	1	0	0	0	0	0
2-6 yrs.	12	1	15.00	54	12	22.22	21	0	2	0	0	2	9.52
7-11 "	40	6	15.00	229	34	14.85	94	1	6	7	4	18	19.15
12-16 "	81	5	6.17	285	30	10.53	310	1	6	24	8	39	12.58
17-21 "	258	12	4.65	205	16	7.80	543	4	5	27	6	42	7.73
22-26 "	253	9	3.56	125	7	5.60	458	5	4	14	2	25	5.46
27-31 "	173	7	4.05	94	15	15.96	298	3	2	9	0	14	4.70
32-36 "	121	9	7.44	55	4	7.27	215	4	3	14	3	24	11.16
37-41 "	107	5	4.67	28	5	17.86	162	1	2	5	1	9	5.56
42-46 "	79	1	1.27	20	3	15.00	107	0	2	2	2	6	5.61
47-51 "	37	1	2.70	3	0	57	1	1	2	0	4	7.02
52-56 "	27	0	3	0	30	0	0	0	0	0
57-61 "	11	1	9.09	2	0	13	0	0	1	0	1	7.69
62-66 "	5	0	1	0	6	0	0	0	0	0
67-71 "	2	0	0	0	2	0	0	0	0	0
Total	1207	57	4.72	1110	127	11.44	2317	20	33	105	26	184	7.94

* H: histological examination + or -.
Z: removed contents examined + or -.

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it occurred in the 7 to 11 year group. It is admitted that in our series the number of cases in the younger group is small. However, Cram and associates,²² by anal swab examination, found the incidence to be higher in the school age (6 to 18 years, 50.3 per cent) than among pre-school children (2 to 5 years, 28.9 per cent). This lower incidence among pre-school children was also

TABLE II
Incidence of Appendiceal Oxyuriasis Among White Beneficiaries of the United States Public Health Service Hospitals

Age groups (years)	Male		Female		Both sexes		
	No.	With oxyurids	No.	With oxyurids	No.	With oxyurids	Percentage positive
2-6	1	0	1	0	2	0	...
7-11	2	0	4	0	6	0	...
12-16	6	0	13	2	19	2	10.53
17-21	161	17	27	1	188	8	4.26
22-26	146	2	22	0	168	2	1.19
27-31	90	2	24	0	114	2	1.75
32-36	77	4	13	0	90	4	4.44
37-41	75	2	11	1	86	3	3.49
42-46	62	1	3	1	65	2	3.08
47-51	22	0	5	0	27	0	...
52-56	19	0	2	0	21	0	...
57-61	8	0			8	0	...
62-66	3	0			3	0	...
67-71	2	0			2	0	...
Total	674	18 (2.67%)	125	5 (4.00%)	799	23	2.88

TABLE III
Incidence of Appendiceal Oxyuriasis Among Indians of the United States

Age groups	Male		Female		Both sexes		
	No.	With oxyurids	No.	With oxyurids	No.	With oxyurids	Percentage positive
0-23 mos.	1	0			1	0	...
2-6 yrs.	10	1	7	0	17	1	5.88
7-11 yrs.	36	6	49	12	85	18	21.18
12-16 yrs.	74	5	204	30	278	35	12.59
17-21 yrs.	81	4	248	26	329	30	9.12
22-26 yrs.	95	6	166	12	261	18	6.90
27-31 yrs.	63	2	92	5	155	7	4.52
32-36 yrs.	40	4	74	12	114	16	14.04
37-41 yrs.	23	3	41	2	64	5	7.81
42-46 yrs.	9	0	22	3	31	3	9.68
47-51 yrs.	12	1	14	3	26	4	15.38
52-56 yrs.	6	0			6	0	...
57-61 yrs.	2	1	2	0	4	1	25.00
62-66 yrs.	2	0	1	0	3	0	...
Total	454	33 (7.27%)	920	105 (11.41%)	1374	138	10.04

CARDIAC LESIONS RESEMBLING ASCHOFF BODIES IN MICE *

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(From the Laboratories of the Mount Sinai Hospital, New York, N. Y.)

In a previous paper we ¹ have described lesions similar to the Aschoff nodules of human rheumatic fever, occurring spontaneously in the hearts of rabbits. This paper deals with spontaneous carditis observed in mice.

Mice used in this investigation received intracerebral inoculations with material from the hearts of patients who had succumbed to acute rheumatic fever. The mice were 40 to 50 days old. A number of the Swiss strain ² were included and a further group was exposed to X-ray irradiation ³ in an effort to decrease the resistance to virus infection. Neither factor proved important in the results which are to be discussed. Of 203 injected mice, cardiac lesions of varying severity were found in 60 (29.5 per cent). The intensity of the process was arbitrarily graded from 0 to 4 plus. Table I shows the frequency of mild and severe lesions.

TABLE I
Incidence and Degree of Severity of Lesions

	Injected mice		Control series	
	No.	Per cent	No.	Per cent
No lesions	143	70.5	43	67.2
Lesions++++	7	3.5	4	6.3
Lesions+++	11	5.4	2	3.1
Lesions++	21	10.3	6	9.4
Lesions+	21	10.3	9	14.0
Total	203		64	

As in the experiments dealing with rabbits,¹ an attempt was made to relate these lesions to the inoculation. This was futile. In 64 control mice the incidence of cardiac lesions (32.8 per cent) approximated that in the injected group. Data for this group also appear in Table I. Since there were no significant differences in either the total incidence or the severity of the process between the inoculated and control groups, it was necessary to conclude that the cardiac lesions had occurred spontaneously in both. We

* Received for publication February 10, 1941.

found by Kuitunen-Ekbaum²³ and Chanco and Soriano.²⁴ Goodale,¹⁷ studying appendices, found for males a higher rate of infestation in the 11 to 20 year group than in the 1 to 10 year period, and the reverse for females. His figures cannot be broken down further for a more direct comparison as to age incidence. Considering the large number of reports on incidence of appendiceal oxyuriasis, it is surprising that so few give consideration to infestation rates by ages. Harris and Browne,¹⁵ Beck¹⁶ and Warwick¹⁹ give ages only for the positive cases.

TABLE IV
Incidence of Appendiceal Oxyuriasis Among Eskimos and Aleutians

Age groups (years)	Male		Female		Both sexes		
	No.	With oxyurids	No.	With oxyurids	No.	With oxyurids	Percentage positive
2-6			1	1	1	1	100.00
7-11	2	0	1	0	3	0
12-16	1	0	11	2	12	2	16.67
17-21	6	1	10	3	16	4	25.00
22-26	6	1	16	4	22	5	22.73
27-31	9	3	9	2	18	5	27.78
32-36	2	0	6	3	8	3	37.50
37-41	2	0	3	1	5	1	20.00
42-46	1	0	3	1	4	1	25.00
47-51	1	0	1	0	2	0
52-56	1	0			1	0
Total	31	5 (16.13%)	61	17 (27.87%)	92	22	23.91

Gordon⁸ pointed out that it is necessary to correct for age in comparing incidence of appendiceal oxyuriasis between the sexes. He found that for the ages 2 to 46 years, the ratio of positive cases was 1 male to 1.4 females. If his figures are examined in small groups (5 year intervals), there is a considerable variation of this ratio (Table V) ranging from 1 male to 0.81 females, to 1 male to 3.74 females. In our study a similar variation occurs, though to much less extent. The lowest ratio, 1 male to 1.33 females, occurs in the 2 to 6 year group, and the highest, 1 male to 2.41 females, is seen in the 12 to 16 year age period. The ratio of the positives is 1 male to 2.34 females when all cases between 2 and 46 years are included.

This higher incidence in females has been reported by many authors including Fischer,⁴ Harris and Browne,¹⁵ Goodale,¹⁷

could not demonstrate, however, an acute epizootic such as was described among the rabbits suffering cardiac lesions.

Figures 1 to 8 illustrate the varied pathological findings, comprising in some animals a true pancarditis; with verrucous involvement of the valves; a mononuclear pericarditis; myocardial foci, often perivascular and containing "owl-eyed" cells resembling those seen in human rheumatic fever; and in other animals showing varying degrees of arteritis and periarteritis.

DISCUSSION

Pathological examination disclosed the following similarities in the spontaneous lesions of the rabbit and the mouse, and the rheumatic human lesions: In all three a focal necrosis of the myocardium was noted. The foci tended to be perivascular. The replacement reaction was mononuclear in type with a tendency to "owl-eyed" cells (which some consider pathognomonic of human Aschoff bodies). In the hearts of all three species, endocarditis, valvulitis, pericarditis and arteritis were also observed. In the more advanced lesions reduplication of the supporting tissue of the endothelium was demonstrated by connective tissue stains. In all three, bacteriological cultures with refined aërobic and anaërobic technics failed to disclose bacteria. Specially stained preparations of the heart lesions also proved devoid of organisms. In none of the species has the etiology of the carditis been determined.

For the study of certain human diseases of unknown etiology, observations of similar spontaneous syndromes of lower animals offer an approach to an understanding of the pathology of the human disease. Smith⁴ demonstrated the importance of this method. Recently, studies of this sort have given interesting results in the work of Shope⁵ and others on the relationship of human and animal influenza. From this point of view interest may be justified in the description of a disease in mice which resembles in form, if not in etiology, the rheumatic pancarditis of man.

CONCLUSIONS

1. A spontaneous pancarditis, resembling human rheumatic pancarditis, is described as it has been observed in mice.
2. The implications of this observation from the viewpoint of comparative pathology are discussed.

7:30 p.m.: 1 cc. cord suspension from rabbit No. 16 instilled into each nostril.

8:10 p.m.: t. 102°.

8:40 p.m.: blood sugar, 60 mg.

9:00 p.m.: 1 cc. cord suspension instilled into each nostril.

10:00 p.m.: t. 100.9°.

10:55 p.m.: t. 101.5°.

11:45 p.m.: t. 101.6°; blood sugar, 97 mg.

4/2/39. 3:20 p.m.: t. 104°; blood sugar, 120 mg.; insulin, 1.7 unit.

5:00 p.m.: 1 cc. cord suspension instilled into each nostril.

5:10 p.m.: blood sugar, 55 mg.

5:40 p.m.: t. 101.2°.

6:30 p.m.: t. 99.6°.

6:40 p.m.: blood sugar, 20 mg.

6:45 p.m.: convulsions; glucose administered.

6:55 p.m.: rabbit fully recovered.

7:20 p.m.: 1 cc. cord suspension instilled into each nostril.

7:45 p.m.: t. 98.4°.

9:10 p.m.: t. 100.4°.

4/3/39. t. 104.0°; no paralysis.

4/4/39. Died.

Autopsy: Cord hemorrhagic and soft. Microscopic examination showed scattered severe neuronal necrosis at various levels (Fig. 4).

III. Controls.

A. INSULIN ALONE. Several rabbits dying in insulin shock showed no neuronal changes.

B. MONKEY VIRUS SUSPENSIONS ALONE. No lesions were observed after intracerebral injection.

C. INNOCUOUS MONKEY CORD SUSPENSIONS AND INSULIN. Sixteen rabbits were injected intracerebrally in this group. There were no spontaneous deaths and no lesions were found on microscopic examination.

D. NORMAL RABBIT CORD SUSPENSION AND INSULIN. No deaths and no lesions were found on microscopic examination.

E. INOCULATION OF MONKEYS WITH SUSPENSIONS OF RABBIT CORD VIRUS.

REPRESENTATIVE PROTOCOLS

Monkey No. 3.

MV virus

↓ (intracerebrally)

Rabbit No. 13

↓ (intracerebrally)

Monkey No. 3

3/12/39. t. 102.5°; injected with cord suspension from rabbit No. 13.

3/13/39. t. 102.0°.

3/14/39. t. 102.0°.

3/15/39. t. 103.5°.

3/16/39. t. 102.0°.

3/17/39. t. 103.5°.

3/18/39. t. 102.0°.

3/19/39. t. 102.5°.

3/20/39. t. 105.7°; tired easily; respirations rapid.

3/21/39. t. 104.4°; tremors; excited.

3/22/39. t. 100.0°; hair ruffled; marked tremors; paralysis of left lower extremity.

3/23/39. t. 100.5°; paralysis of both lower extremities.

3/24/39. t. 100.5°; paralysis of all extremities; killed.

Autopsy: Cord hemorrhagic and markedly edematous. Microscopic examination showed interstitial and perivascular infiltrations, and severe necrosis of anterior horn cells (Fig. 5).

Monkey No. 4.

MV virus

↓ (intracerebrally)

Rabbit No. 13

↓ (intracerebrally)

Rabbit No. 16

↓ (intranasally)

Rabbit No. 19

↓ (intranasally)

Monkey No. 4

4/24/39. t. 102.8°; inoculated intranasally with cord suspension from rabbit No. 19.

4/25/39. t. 101.8°.

4/26/39. t. 102.0°; hair ruffled; less active; left upper extremity appeared limp.

4/27/39. t. 100.6°; cry was weak; left upper extremity improved.

4/28/39. t. 100.6°.

4/29/39. t. 99.4°.

4/30/39. t. 101.0°.

5/1/39. t. 102.8°.

5/2/39. t. 102.6°; left upper extremity not used as often as the right, and was weaker than the right.

5/3/39. Killed.

Autopsy: All cut surfaces of cord showed pin point hemorrhages, more marked on left side of cord in the cervical region. Microscopic

3. The discovery of these lesions in control animals will prove to be of value to pathologists who attempt to evaluate experimental cardiac lesions in mice.

NOTE: We wish to thank Paul Klemperer and Gregory Shwartzman for their interest and helpful suggestions. We are also deeply indebted to the late Louis Gross for the time which he devoted to the pathological aspect of this work. Impressed with the resemblance of certain lesions to the Aschoff bodies in rheumatic myocarditis, he applied to them the term "Aschoffoid."

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ANTERIOR AND POSTERIOR RHACHISCHISIS *

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Two fetuses exemplifying anterior and posterior rhachischisis have appeared in the embryological collection of the School of Medicine of West Virginia University. Both show clearly the anomaly of the vertebral column, together with the characteristic complex of visceral anomalies associated with it. The condition must be rather rare; I have found reference to only thirty cases in the literature. Yet, it cannot be dismissed casually as a developmental curiosity because it involves an extensive complex of anomalous characteristics, which, in all recorded cases, run true to type. For this reason, it seems worth while to describe the two cases, together with notes on earlier records and comments upon the probable mode of development.

REPORTS OF CASES

Case 1

Male fetus (No. 121) had a menstrual age of 8 months and weighed 2.3 Kg. The head measured 42.5 cm. in circumference. The neck was very short and thick (Figs. 1 and 2). It had club feet (talipes varus), and there were in each jaw four completely erupted incisor teeth. It was secured through Dr. W. B. Scherr, of Morgantown, W. Va.

The Skull. The foramen magnum was unusually large, measuring 27 by 24 mm. (Figs. 2 and 3). Otherwise the skull showed no conspicuous anomalies, except a separation of the bones of the skull cap, characteristic of hydrocephalic infants.

The Vertebral Column. The outstanding anomaly was the complete division into right and left halves of all the cervical and the first seven thoracic vertebrae (Figs. 3 and 5). The two halves of the divided vertebral column were widely separated in their mid-portion, due to strong lateral curvature, so that they bounded a somewhat circular opening about 2 cm. across, lying just below the foramen magnum (Fig. 3). Both halves of the vertebral column also showed strong lordosis (Figs. 4 and 6). The divided vertebrae were decidedly shorter than the corresponding vertebrae

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DESCRIPTION OF PLATES

Hematoxylin and eosin staining was used throughout.

PLATE 134

FIG. 1. Mouse No. 198. A lesion in a sinus of Valsalva adjacent to the valve simulates an Aschoff nodule. Interstitial valvulitis is present, most marked at the base. The myocardium shows proliferation of "myocytes," histiocytes with an occasional giant cell and lymphocytes. There is destruction of muscle fibers. $\times 275$.

FIG. 2. Mouse No. 198. Higher magnification of lesion shown in Figure 1. The nodular infiltration consists predominantly of polygonal, often stellate, mononuclear cells. These have large pale nuclei with conspicuous nucleoli, and basophilic cytoplasm. $\times 625$.

in a normal fetus. These three factors were responsible for the great shortening of the neck. Each half vertebra had a well developed half arch and half body, between which, on the medial surface, was a notch representing a half intravertebral foramen.

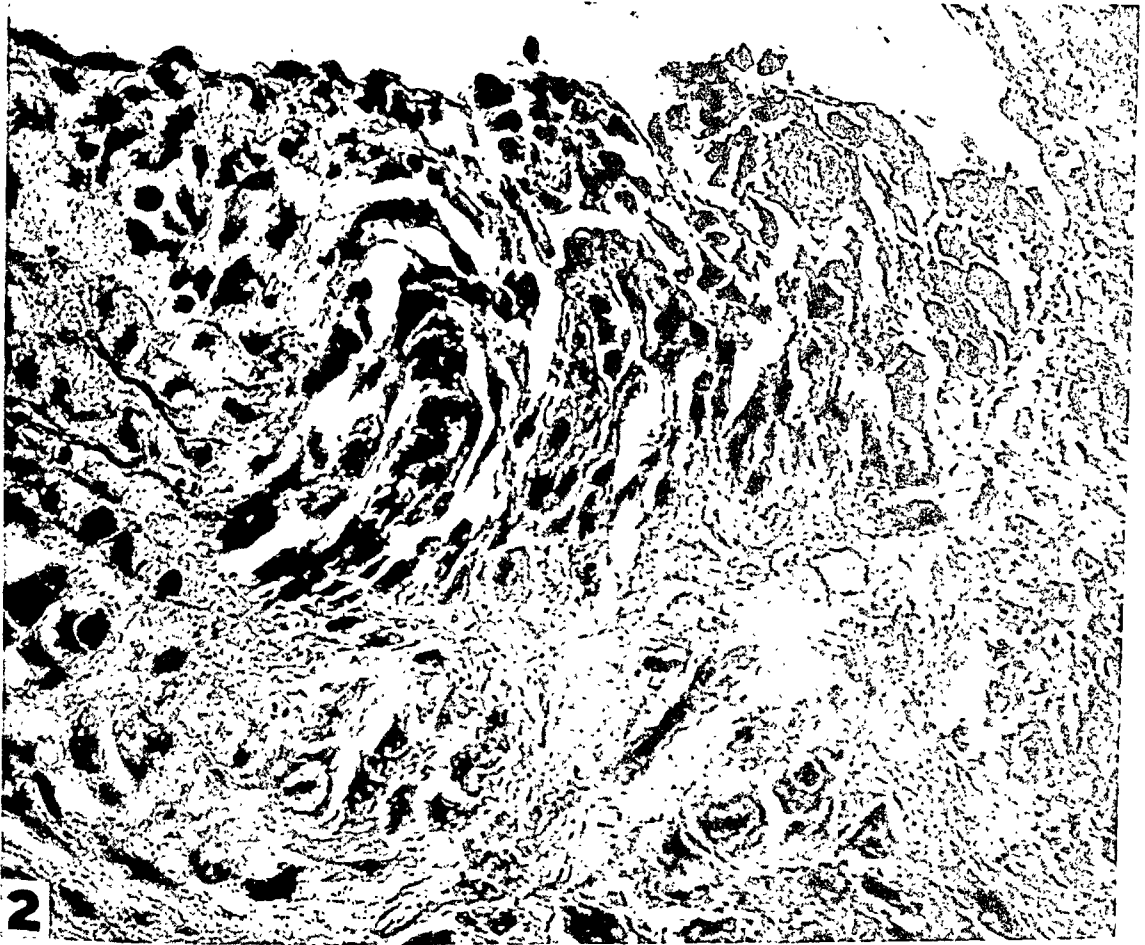
The Central Nervous System. The brain was typical for hydrocephalic infants, with very large lateral ventricles enclosed by greatly distended, thin walls (Fig. 2). The spinal cord was not divided in the region of the divided vertebral column, but was expanded laterally to form a discoid enlargement occupying the circular opening between the two halves of the vertebral column. The spinal nerves radiated from this expansion to their respective vertebral foramina.

The Thoracic and Abdominal Viscera. The viscera showed conspicuous anomalies characterized by extensive ectopia of abdominal organs into the thorax (Fig. 2). On the right side, the diaphragm was complete except for a slit about 1 cm. long. The left side was practically without diaphragm. The pericardium had, on the left side, a hole about 1 cm. across. Thus, pleural, pericardial and peritoneal cavities were in communication with each other.

The heart and lungs were at their usual level above the diaphragm, but lay wholly to the right of the mid-plane of the body and in the ventral half of the thorax. The mediastinum lay obliquely, with its ventral and caudal margins well to the right of the mid-plane. The stomach and the greater part of the small intestine lay in the right pleural cavity, occupying the dorsal half of the right side of the thorax. The stomach was partly in the mediastinum and partly attached to its right side. The esophagus was short. At a little distance above the level of the diaphragm, the small intestine crossed from the right to the left side of the body, and thence coursed to the lower abdominal region. The large intestine was poorly developed.

The left pleural space was occupied largely by about one-third of the liver which entered through the opening in the diaphragm. The spleen lay in the extreme cephalic end of the left pleural space, attached to the left side of the mediastinum. A narrow spatulate lobe of the liver entered the right pleural cavity through the small slit in the diaphragm.

The great omentum was present as a serous sac lying between



the stomach and the right lung. Its attachments were to the right side of the mediastinum and to the greater curvature of the stomach. Otherwise, the serous relations of the abdominal viscera were so distorted that they cannot be designated in conventional terminology.

Case 2

Full-term female fetus (No. 53) weighed 4.3 Kg. It was secured through Dr. A. D. Hunger, of Point Marion, Pa. In all essential features of the complex of anomalies, it was similar to case 1, differing only in details and in external appearance. The head was of normal size. The neck was very short and thick. From the lower occipital region and the short neck there arose a large, pendulous, skin-covered meningeal sac about 12 cm. long and 10 cm. wide (capacity about 200 cc.), hanging to about the tenth thoracic segment (Fig. 7).

The Skull. The skull had no evident gross anomalies, except that the foramen magnum was very large, measuring 4.7 cm. in anteroposterior diameter by 3.0 cm. in width (Figs. 7 and 9). Its unusual expansion was in dorsal and lateral directions. Its ventral edge was in the usual position. Its ventral portion communicated with the spinal canal; its dorsal portion opened above the vertebral column, and through it the meningeal sac protruded (Fig. 7).

The Vertebral Column. There was complete division into right and left halves (as in case 1) of all cervical and the first thoracic vertebrae. The two halves were divergent and well separated, leaving between them a somewhat circular opening about 1 cm. in diameter (Fig. 8). There was lordosis in this region and the individual vertebrae were not of normal length.

The Central Nervous System. The brain was of about normal size and form. The cerebellum was represented by a disintegrating mass, of about the usual size for the organ, enclosed by a meningeal covering. It protruded by about half its diameter through the upper part of the foramen magnum into the meningeal sac (Fig. 7). The cervical spinal cord was conspicuously malformed in that it simulated somewhat the cross-sectional form of the medulla oblongata, with a thick floor of nervous tissue and a thin, broad roof of non-nervous tissue. In fact, the medulla oblongata and the cervical portion of the cord together appeared like an extended medulla. The fourth ventricle was directly continuous, without decrease in size, with the greatly expanded central canal of the cervical cord, forming a continuous cavity about

PLATE 135

FIG. 3. Mouse No. 40. Bland verrucous lesion, covered with endothelium, at the root of the aorta in the sinus pocket. $\times 525$.

FIG. 4. Mouse No. 151. Acute arteritis and periarteritis. Periarteritis nodosa-like, vascular lesion. $\times 450$.

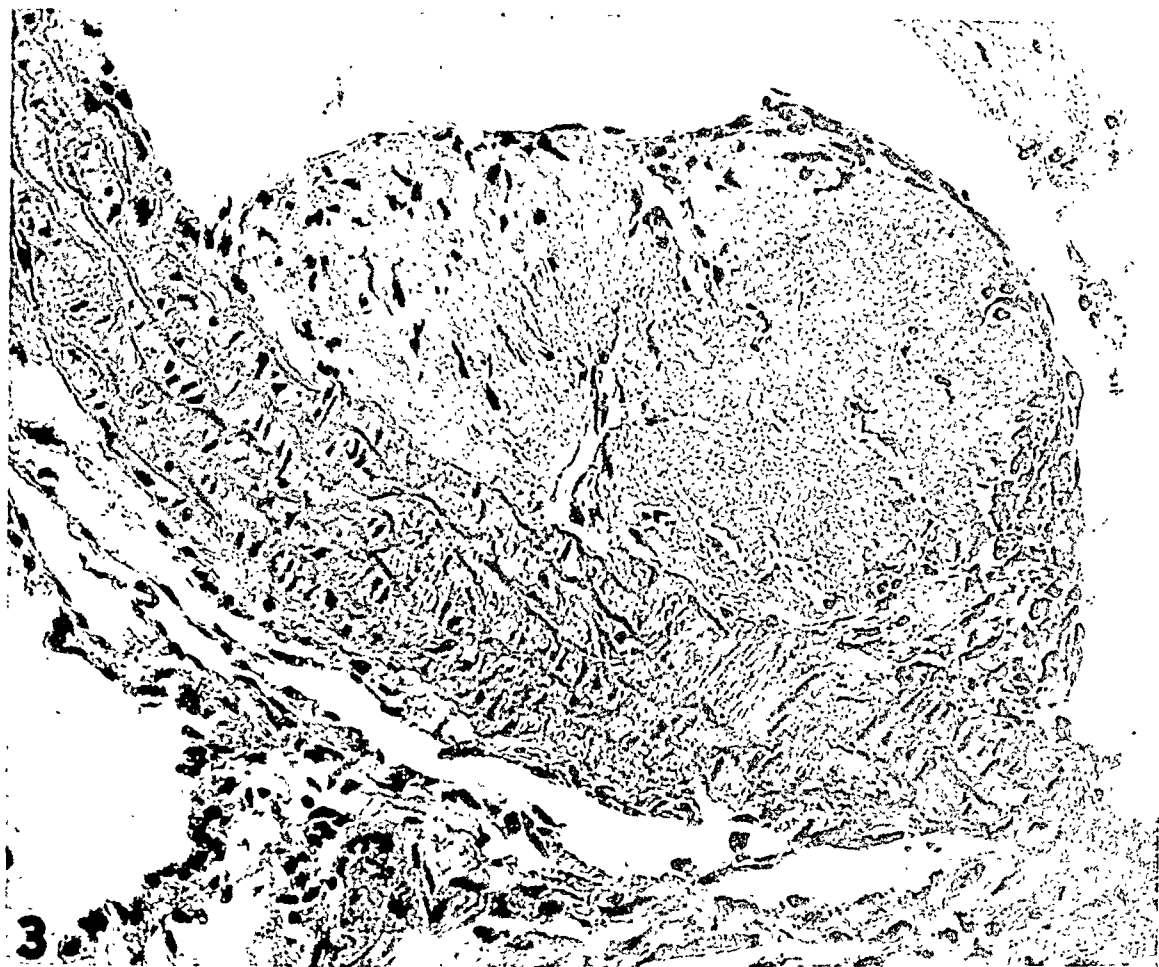
1.5 cm. wide by 2.5 cm. long. The actual boundary between medulla and cord was at about the usual level with reference to the foramen magnum, as indicated by spinal and cranial nerves and as shown by microscopic sections.

It was from the thin roof of this part of the cord, at about the level of the fourth cervical nerves, that the meningeal sac arose, by a passage measuring about 1.5 cm. in lateral extent by 0.5 cm. in height (Fig. 7). The sac was covered externally by skin, inside which were the spinal meninges. Though microscopic sections were studied from different regions of the sac as well as from the cervical spinal cord, it was not determined with certainty whether the layer lining the sac was an extension of the roof plate of the spinal cord or was derived from the meninges.

There was an anomalous filament of nervous tissue, about 5 mm. long by 0.5 mm. thick, extending ventrad from the floor of the cervical cord at about the level of the fourth cervical nerves. It occupied the center of the circular opening between the two halves of the vertebra. Its basal portion was of gray matter, continuous with the gray matter of the cord; from its more distal portion arose several groups of medullated axons which could not be traced far. The possible significance of this structure will be discussed later.

The Thoracic and Abdominal Viscera. The anomalies of these organs (Fig. 7) were of the same general nature as in case 1. The diaphragm was almost wanting on the left side, but was complete on the right. The pericardium was incomplete on the left side, having a hole about 1.5 cm. in diameter. The right pleural sac was normally closed, but the left pleural sac, the pericardial cavity and the abdominal cavity were in free communication.

The heart and lungs were at the usual level above the diaphragm, but were displaced, together with the mediastinum, into the right side of the thorax. The spleen was high in the left side of the thorax. About one-third of the liver extended into the left thorax through the large opening in the diaphragm. The stomach was just above the level of the diaphragm; the esophagus reached the stomach through the posterior mediastinum, but entered it on its caudal side; the small intestine (165 cm. long), except for the pyloric end of the duodenum, lay in the abdominal region. The large intestine was well developed (about 20 cm. long) and ab-



normally placed, in that from its origin in the lower left abdominal region it coursed almost directly cephalad to the upper end of the thorax and thence directly caudad to the anus. The ectopic abdominal organs occupied not only the left side of the thorax, but had encroached to a considerable extent upon the right side, leaving much less than half the thoracic space for the usual thoracic organs.

The great omentum was recognizable as a small serous sac above the diaphragm with connections to stomach, spleen and large intestine. The mesentery of the small intestine was of about normal form, but the other serous connections of the alimentary organs were greatly distorted.

DISCUSSION

The Occurrence of the Anomaly

Though *rhachischisis* and *spina bifida* in their ordinary forms are very common anomalies of the vertebral column and spinal cord, the complete division of a series of vertebral segments into widely separated right and left halves is relatively rare.

In *A Reference Handbook of the Medical Sciences** is pictured a vertebral column which shows this anomaly. The illustration is from specimen No. 831 in the Warren Anatomical Museum of the Harvard Medical School. In a personal communication from the curator of the Museum, I have received a brief description of this fetus by Dr. Isaac F. Galloup, 1858. This clearly portrays the anomalies of the vertebrae and viscera which characterize this condition. I have found no other case described in American literature.

Gruber (1926) gives abstracts of 17 cases described by various authors, the earliest in 1824. To these he adds 2 cases from his own observations. Feller and Sternberg (1929 and 1934) add 6 more from the literature and describe 3 others. In all but one of these cases, the cervical and upper thoracic vertebrae were involved, the exception, listed by Gruber (from Reinke, 1877), having the division in the lumbar region. Adelman (1920) describes a similar lumbar anomaly in a calf.

* William Wood & Co., New York, 1923, ed. 4, 7, figure 4552.

PLATE 136

FIG. 5. Mouse No. 171. Marked necrotizing arteritis. $\times 290$.

FIG. 6. Mouse No. 6. Multiple foci of myonecrosis with early scarring.
 $\times 190$.

The General Nature of the Anomaly

It appears, from consideration of the several recorded cases, that the two described herein are typical and that the anomaly is not simple, but includes the following complex of anomalous features:

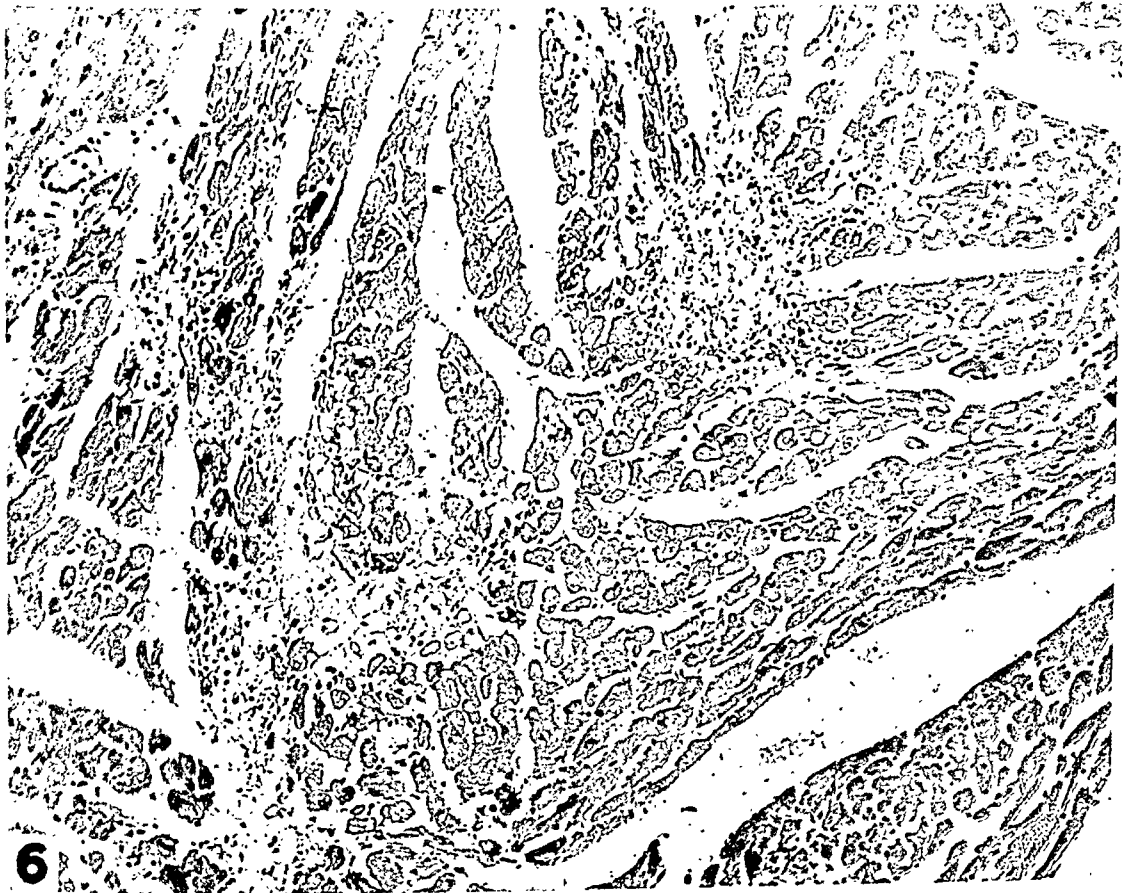
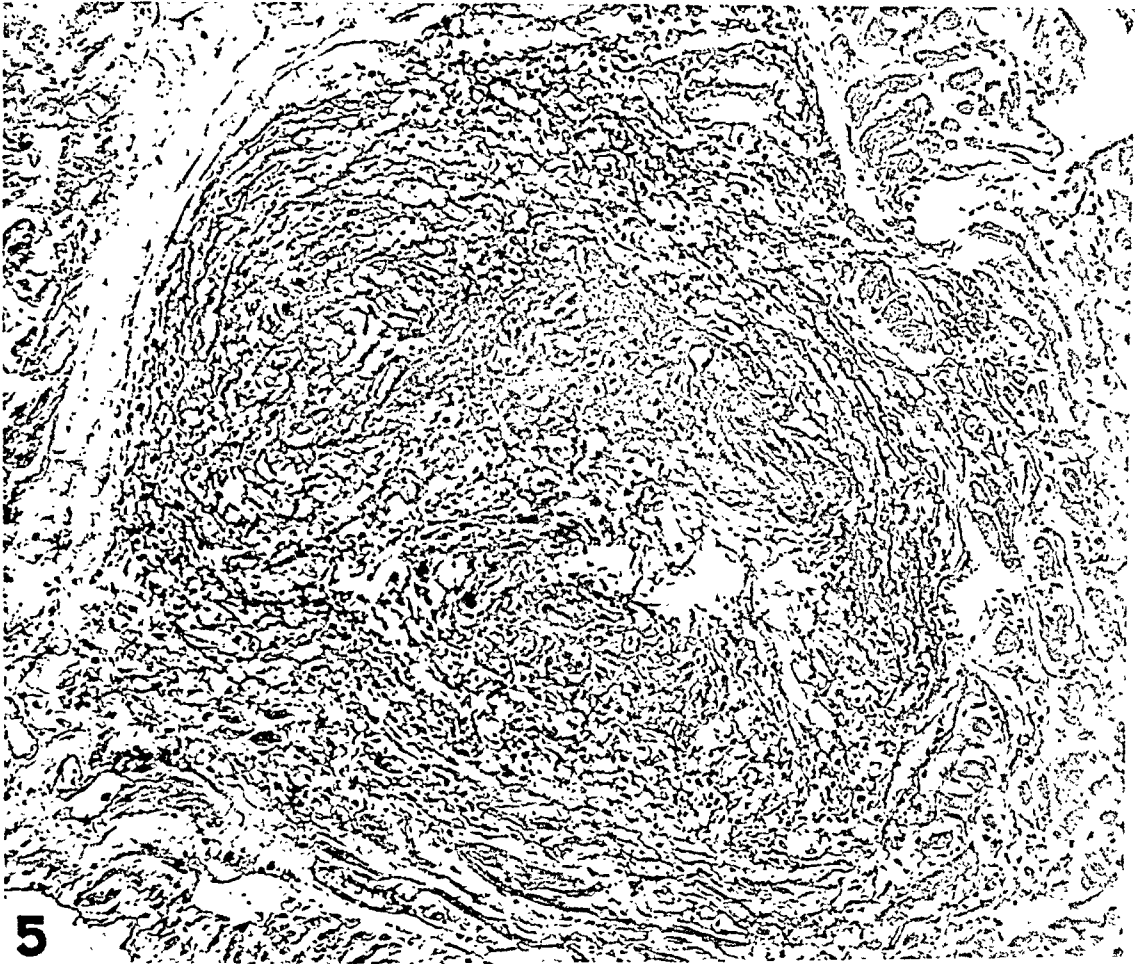
1. The affected vertebrae (usually the cervical and upper thoracic) are completely divided (both arches and bodies) into right and left halves which are widely divergent from each other (Figs. 3, 5 and 8). This condition is fundamentally different from rhachischisis of the ordinary sort in which only the arches are divided.

2. The neck is very short (Figs. 1, 2 and 7). This condition must not be confused with the shortened neck of the ordinary anencephalic or craniorhachischitic fetus, which is due to a quite different defect.

3. In probably all of the recorded cases, there is extensive displacement of abdominal viscera into the thorax (Figs. 2 and 7). The displacement of abdominal viscera into thoracic levels is, in some cases, associated with incompleteness of the diaphragm and the pericardium, while in other cases the diaphragm is complete and the displacement is by way of the posterior mediastinum.

4. Very frequently some division of the alimentary canal is in intimate relation with the spinal cord in the region of the divided vertebrae. Almost any segment of the gut may be involved. Sometimes a loop of the gut passes through the cleft vertebrae and a segment (frequently the stomach) lies dorsad to the vertebral column. There have been observed various forms of patent connections between gut and neural groove, non-patent diverticula and fibrous connections; while some cases show no demonstrable connection of any kind.

5. In the majority of recorded cases, the neural tube is unclosed as in ordinary rhachischisis, giving rise to myeloschisis with its characteristic area medullovasculosa. Various forms of anencephalia and acrania are also of frequent occurrence. But non-closure of the neural tube is not a necessary part of the anomaly, for less frequently there has been recorded complete closure, with the skin completely covering the mid-dorsal region of head and body. The head may be of normal form and size; or it may be hydrocephalic; or there may be meningocele.



6. The spinal cord, or the open neural groove in the affected region, may be either single or divided into right and left halves; or it may be variously deformed.

Ordinary rhachischisis does not involve any accompanying complex of visceral anomalies; anterior and posterior rhachischisis seemingly always involves ectopia of abdominal organs into the thorax, frequently with incompleteness of the diaphragm. This anomaly of the diaphragm should not be confused with simple congenital hernia of the diaphragm, a much more common condition. This latter condition is well illustrated by Liebow and Miller (1940) who also give a good bibliography. In simple congenital diaphragmatic hernia, the distortion of the digestive organs and serous membranes (mesenteries and omenta) is much less radical than in anterior and posterior rhachischisis; and the ectopic abdominal organs frequently may be restored to their abdominal position by operation. The two types, while having much in common, are in reality fundamentally different in their nature and mode of genesis.

Both of the cases here reported exhibit fully the essential features of the anomaly. Both belong to the type with fully formed head and enclosed spinal cord. In neither is the spinal cord divided. In neither is any connection observed between alimentary canal and neural tube, though in case 2 the nervous projection from the ventral side of the cervical spinal cord may be a remnant of a former connection. Both cases belong to the type in which the displacement of abdominal viscera is associated with incomplete diaphragm; the misplaced viscera lie directly within the pleural cavities, not in the posterior mediastinum.

Concerning Terminology

Inasmuch as this anomaly involves an extensive and definite complex of congenital structural defects, it should have a distinctive name. It has frequently been called "spina bifida." But that is hardly a suitable name because, in its strict application, as defined in dictionaries and encyclopedias and as commonly used in textbooks, it designates those conditions in which there is a meningeal protrusion through a rather minor defect in the vertebrae. On the other hand, the application of the term has been so loose and so varied that its use in this case would not be at all defini-

PLATE 137

FIG. 7. Mouse No. 2. Retro-aortic lesion resembling a diffuse Aschoff body.
× 230.

FIG. 8. Mouse No. 233. High magnification of segment of perivascular nodule resembling Aschoff body. Interstitial myocarditis. × 1750.

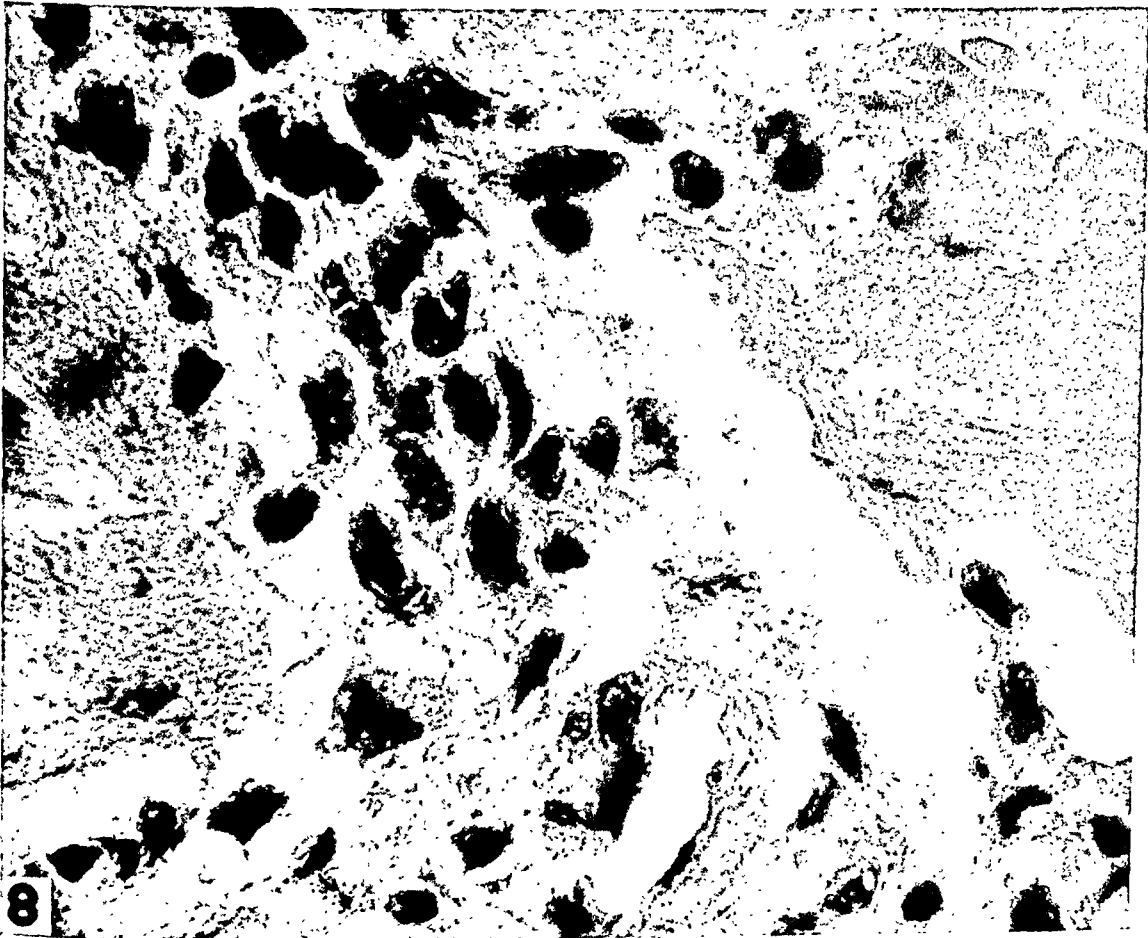
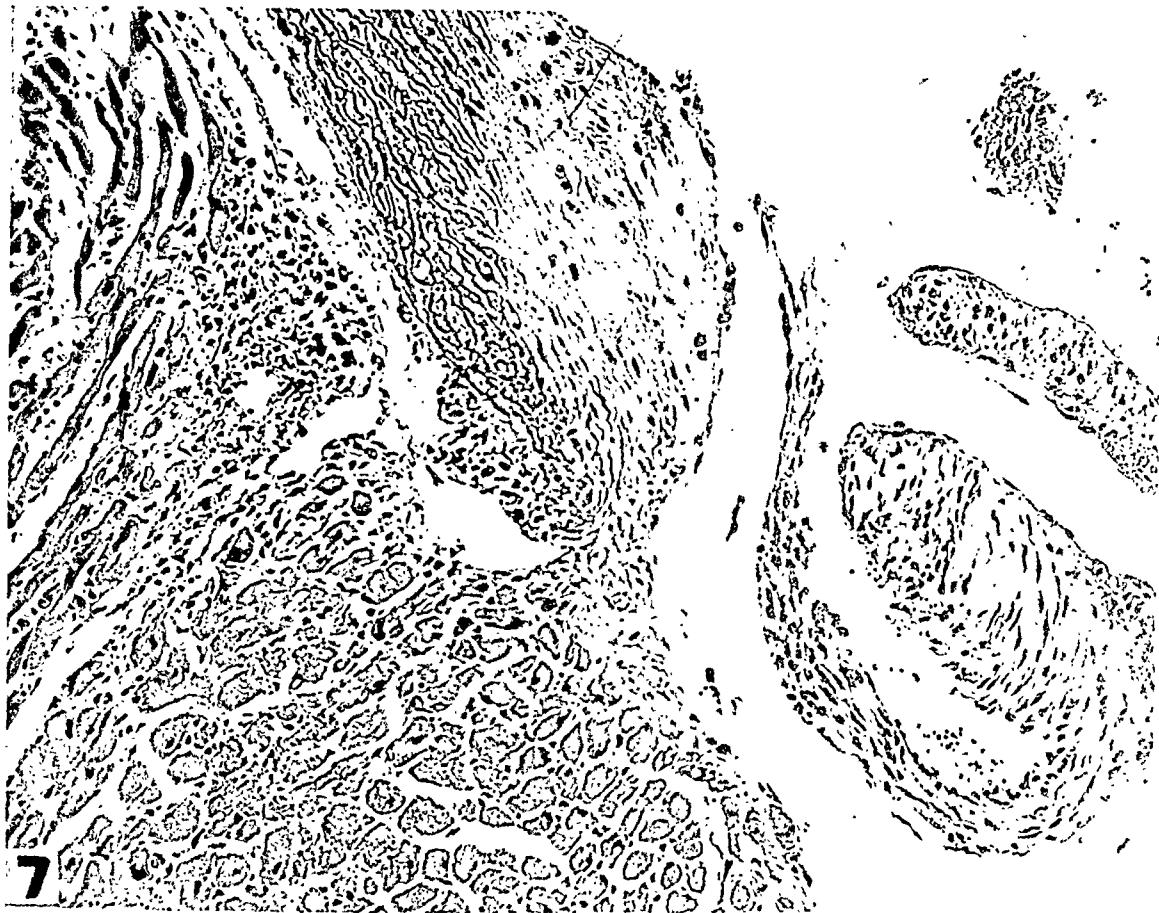
tive. Nor is "rhachischisis" in its ordinary meaning applicable. It has sometimes been designated as "Wirbelkörperspalte," which is a fairly descriptive term, but hardly one which might come into general use. "Vertebra bipartita" has apparently not been used with exactly this application, though it seems suitable.

Probably the best terms (though somewhat cumbersome) are "anterior and posterior spina bifida" and "anterior and posterior rhachischisis." Both of these have been used, and I believe that neither has been applied to any other form of cleavage of the spine. Of the two terms, I have chosen the latter as the more suitable, because "rhachischisis" has not been used with so many different applications as has "spina bifida." In this paper the phrase "anterior and posterior rhachischisis" carries with it not only its primary significance (division of vertebral arches and bodies), but also implies the entire complex of visceral anomalies which seem to be universally associated with it.

The Probable Course of Development of the Anomaly

The common types of spina bifida and rhachischisis result from varying failure of the neural tube to close. On the other hand, anterior and posterior rhachischisis involves failure of the paired sclerotomes to unite, the failure occurring either with or without proper closure of the neural tube. Consequently, this anomaly can in no sense be considered an extreme manifestation of either spina bifida or rhachischisis, but rather an anomaly of a different type.

It would seem, further, that non-fusion of sclerotomes is determined by forking (doubling) of the notochord, inasmuch as notochordal rests have been observed in each half of such vertebral bodies (Feller and Sternberg, 1929). Several writers have contended that the forking of the notochord results from anomalous behavior of the primitive node, and that the anomalous connection, sometimes patent, between gut and neural tube is a persistent neurenteric canal. Adelman (1920) and other writers support this view by reference to Hertwig (1892) and other experimenters who have produced, in amphibian embryos, duplications of axial structures and non-closure of neural tube (all loosely called "spina bifida"). These results were brought about by experimental procedures which were timed to prevent normal development in the region of the blastopore.



Study of these earlier papers shows that the experimental defects produced in amphibian embryos are by no means identical with the human anomalies under consideration. Confusion on this point has arisen because both sets of conditions have been loosely designated as "spina bifida." Moreover, recent experimental work has presented strong evidence that the avian and mammalian primitive streak and neurenteric canal are not derived from the amphibian blastopore. Nevertheless, there are suggestive similarities between the two sets of structures, and recent experimental work gives unexpected support to the view that anterior and posterior rhachischisis may indeed result from anomalous behavior of the primitive node and the neurenteric canal.

According to this recent conception (Jacobson, 1938; Weiss, 1939; and others) the primitive node is a restricted area through which there invaginate two lateral areas of presumptive notochordal material which unite just cephalad to the node to form the definitive notochord. In a somewhat similar manner, the neural plate is formed by the confluence of two lateral areas of presumptive neural material. By this concept, the neurenteric canal, a temporary opening through the node, lies between the two converging streams of notochordal material which normally unite just cephalad to it. So much seems clearly established by experimental procedures.

On this basis, it seems safe to assume that, in rare cases, as an anomaly, an unusually large neurenteric canal, or one which persists too long, might produce doubling of the notochord by preventing the union of the two notochordal streams. Two notochords, separated by a considerable interval, might well prevent the normal union of the paired sclerotome masses, thus producing a series of vertebral bodies about each notochord. The interval of separation would also prevent the union of right and left halves of the neural arches, even though the neural tube might have undergone normal closure. In like manner, the two neural streams might also fail of fusion, thus producing two spinal cords or one malformed cord.

The fact that it is usually in the cervical and upper thoracic regions that such division of vertebrae occurs, correlates well with the probable location of the neurenteric canal during its

normally short existence. There is increasing agreement that the primitive node, at the time of its first appearance, is located in what corresponds to the posterior cephalic region, whence it recedes in a caudal direction as the notochord is spun through it. The human neurenteric canal makes its appearance in the primitive node shortly after the formation of the first pair of somites, and it normally closes after about six pairs have been formed (Bartelmez, 1926, and others). Inasmuch as the somites take definite form at some little distance cephalad to the primitive node, the neurenteric canal, during its period of normal patency, must lie in the cervical or upper thoracic regions, the very levels at which divided vertebrae are common observed. A persistent neurenteric canal is strongly indicated in those cases of anterior and posterior rhachischisis in which there is a patent connection between gut and open spinal cord; a partial persistence is suggested by the diverticula and fibrous connections found in others, or by the nervous appendage observed in case 2.

Since the division of the vertebrae is usually limited to cervical and upper thoracic levels, such anomalous neurenteric canal is clearly left behind in that region instead of receding caudad along with the primitive node. The retention of this union in the cervical region is probably due to attachment to structures which do not normally recede as does the primitive node or the abdominal and thoracic viscera. In the several described cases of anterior and posterior rhachischisis, the peculiar ectopic positions of the abdominal organs are clearly indicative of retention rather than of herniation into the thorax after a normal descent. The anomalous connection between gut and cervical neural tube would prevent the normal recession of other parts of the entodermal tube and its associated mesodermal structures. Faulty development of diaphragm, pericardium and other serous membranes would also result.

On the other hand, it is commonly believed that in simple congenital hernia of the diaphragm, the ectopic abdominal organs have entered the thorax by actual herniation from the abdomen, probably not earlier than the eighth week (Liebow and Miller, 1940). Herein lies a significant distinction between the two types of visceral ectopia.

In general, it would seem that the entire complex of anomalies

examination showed perivascular edema and swelling of vessel walls; focal collections of polymorphonuclears and round cells; cells in the central canal; scattered neuronal necrosis which was most marked in the left anterior horn of the cervical region (Fig. 6).

The severe lesions following nasal instillation of suspensions of virus in hypoglycemic rabbits obviate any possibility that the neuronal changes resulted from intracerebral injections of the material used in the preparation of suspensions. That no spontaneous deaths occurred in the controls injected with insulin and innocuous cord suspensions, and that eight of eleven rabbits injected with insulin and virus suspensions died, indicate that the rabbits in the latter group were infected.

It can be stated with certainty that one to three injections of insulin, using doses of one to two units, will not injure neurons in any way that can be demonstrated histologically, even if the blood sugar is depressed to convulsive level. Rabbits dying in hypoglycemic shock showed normal neurons throughout. Weil, Liebert and Heilbrunn⁹ injected as much as 60 units of insulin into rabbits in one day and were unable to produce demonstrable changes in the neurons. For example, they produced forty-five seizures in one rabbit using a total of 59 units of insulin and found no histopathological changes in the neurons. They found some changes after 70 to 150 units given in divided doses, and severe changes after 200 to 400 units. The significant factor in the production of changes is the cumulative effect of repeated injections of large doses of insulin.

Lesions in the Rabbit. Severe lesions in the medulla and cord may be noted in rabbits dying as early as 13 hours after intracerebral inoculation. The widespread distribution of lesions in such rabbits demonstrates the ability of the virus to invade in a very short time. Apparently the virus encounters little or no resistance during the period of hypoglycemia. Lesions are distributed unevenly much as they occur in humans and monkeys. One side of the cord may show more marked involvement than the other. Lesions may be present in the cervical and lumbar regions and absent in the thoracic. The presence of neuronal injury is the striking feature of the pathological picture. Capillary, arterial, and venous engorgement is prominent, accounting

for the hyperemic appearance of the cord on gross examination. Neuronophagia was not observed. Perivascular and interstitial infiltrations were absent. In mild injury the neurons appear swollen, the Nissl bodies are less sharply defined, and the cell as a whole takes a lighter and more eosinophilic stain. In moderate injury the neurons are pale and swollen and present a "washed-out" appearance with absence of Nissl bodies; the nuclei are clearer, granular, and may contain inclusion bodies. In severe injury the neurons are shrunken, with pyknotic and fragmented nuclei; the cytoplasm is undergoing dissolution and in some instances is barely distinguishable from the ground substance. Often a mass of debris represents the site of the neuron and sometimes it may completely disappear.

The presence, exclusively, of neuronal injury and the absence of perivascular and interstitial infiltrations represent the fundamental nature of infection with a neurotropic virus. Hurst¹⁰ has emphasized the fact that the injury to the neuron is the essential lesion in a neurotropic virus disease such as poliomyelitis. The following is quoted from his article: "In all probability viruses are obligatory intracellular parasites. If, therefore, the adjective neurotropic as applied to certain viruses has any significance, we should logically expect such viruses to be capable of a primary and direct attack on the nerve cells. Such is in fact the case. Yet the older literature on so typical a neurotropic virus disease as poliomyelitis ascribed the lesion in nerve cells wholly or in large part to impaired nutrition, resulting from interstitial inflammation . . . The microscopical appearances of nerve cells affected by a virus vary with the acuteness or otherwise of the disease process. In hyperacute and acute conditions, and hence commonly in experimental infections . . . the type lesion is acute necrosis of the neurons. The cytoplasm is shrunken and strongly eosinophilic, the nucleus pyknotic, karyorrhectic, fading or absent; in the brief pre-necrotic phase various types of neuronal change described by Nissl may be seen, but especially the so-called ischemic cell-change and the 'severe cell-change'."

Course of the Disease in Rabbits. The animal may show evidence of infection in 8 to 10 hours after intracerebral inoculation. After nasal inoculation signs of infection may not be evident until

finds its most reasonable explanation in anomalous behavior of the neurenteric canal, an explanation which serves to tie together the striking anomalies of skeleton, nervous system and body viscera.

SUMMARY

The two fetuses described in this paper had the following anomalies which are characteristic of the general complex designated here as "anterior and posterior rhachischisis":

1. The neck was very short and thick.
2. The shortness of the neck was due to the complete division into right and left halves of all of the cervical vertebrae and some of the thoracic vertebrae.
3. The diaphragm was practically wanting on the left side. There was a good-sized opening in the left side of the pericardium between the pericardial cavity and the left pleural cavity.
4. A large portion of the liver, the spleen and portions of the abdominal alimentary tract were in the thorax, crowding the heart and lungs wholly to the right side of the median plane.

Each fetus had other external and internal anomalies which are not considered as part of the general complex comprising anterior and posterior rhachischisis.

There is included a discussion of about thirty previously described cases, together with comments upon opinions of earlier writers as to the mode of origin of the anomaly, interpreted in the light of certain recent experimental embryological work. Anterior and posterior rhachischisis is compared with the ordinary forms of spina bifida and rhachischisis, and with the common congenital diaphragmatic hernia.

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turity of the resulting granuloma in a child, aged 33 days, was such as to indicate that the process was congenital.

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DESCRIPTION OF PLATES

PLATE 138

- FIG. 1. Case 1. External form. Photograph after preservation in solution of formaldehyde.
- FIG. 2. Case 1. Photograph after sectioning in the sagittal plane, showing the absence of neck, the expanded skull and the distended cerebral hemispheres characteristic of hydrocephaly, the somewhat enlarged foramen magnum, the spinal canal (spinal cord removed) connecting with the foramen magnum, the undivided lower thoracic vertebrae, the divided upper thoracic and cervical vertebrae (the divided vertebrae are not seen in the plane of this section on account of their strong lateral curvature as shown in Fig. 5), the large liver partly in the thorax, the incomplete diaphragm partly subdividing the liver, and the stomach lying between the thoracic liver and the vertebral column.
- FIG. 3. Case 1. Dissection to show division of vertebral column in an oblique view. The two halves of the divided vertebrae bound a roughly circular opening through which are seen the base of the skull and the foramen magnum. The expanded portion of the spinal cord occupied this opening, giving off nerves radially through foramina in the encircling half vertebrae.
- FIG. 4. Case 1. Lateral view after dissection. The strong lordosis of the divided part of the vertebral column and the pronounced lateral curvature (see Fig. 3) bring the base of the skull close to the upper thoracic vertebrae. Shortening of the segments in the divided region is also shown.

DESCRIPTION OF PLATE

PLATE 140

- FIG. 1. Low power photomicrograph through the spinal cord at a lumbar level showing the granuloma in its dorsal position. Hemalum and eosin stain. $\times 40$.
- FIG. 2. Higher power photomicrograph through a portion of the granuloma seen in Figure 1 showing two hairs in longitudinal section and many others in transverse section surrounded by giant cells. Hemalum and eosin stain. $\times 300$.
- FIG. 3. High power photomicrograph showing multinucleated giant cells plastered about segments of hair seen in cross section. Hemalum and eosin stain. $\times 700$.



Dodds

Anterior and Posterior Rhachischisis

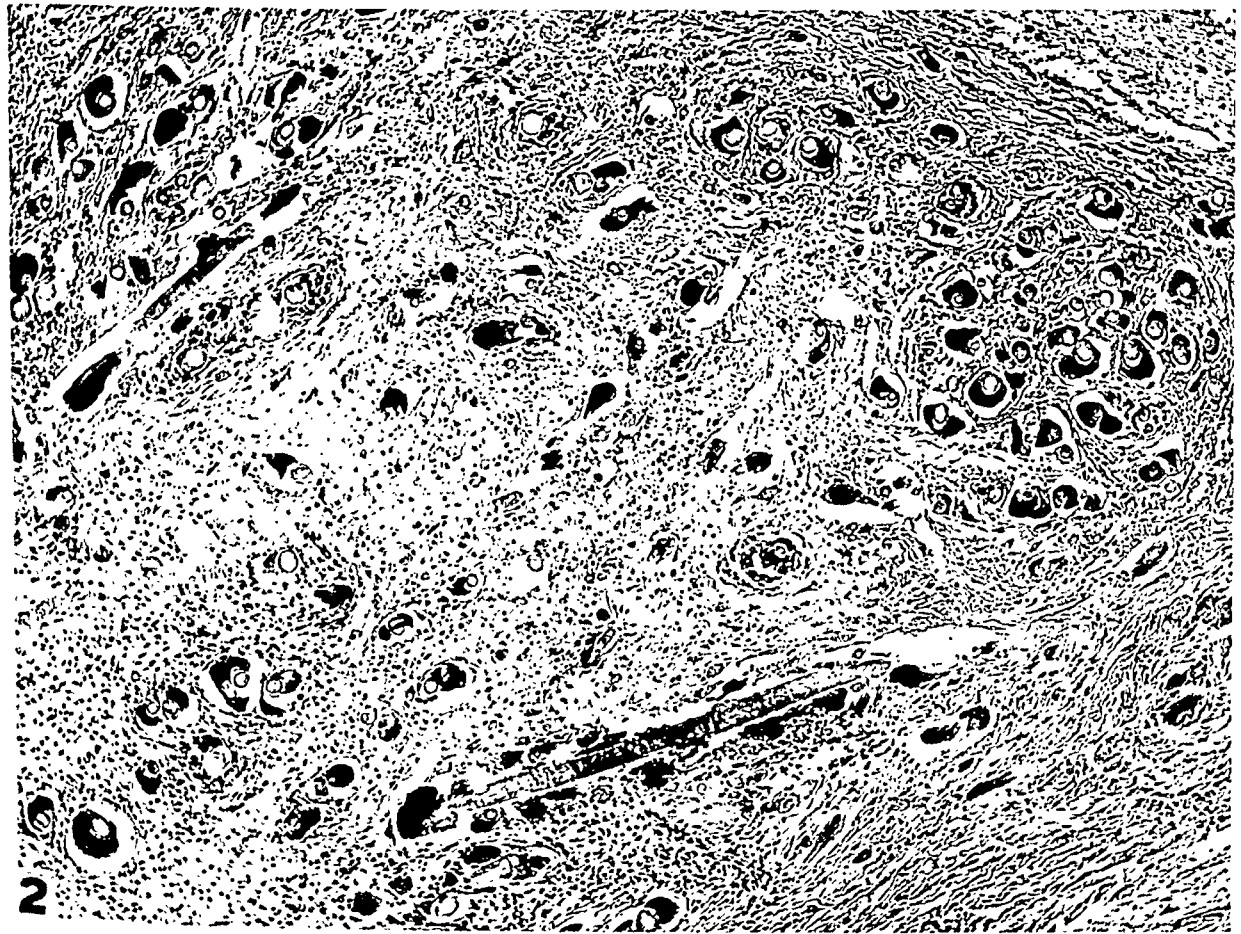
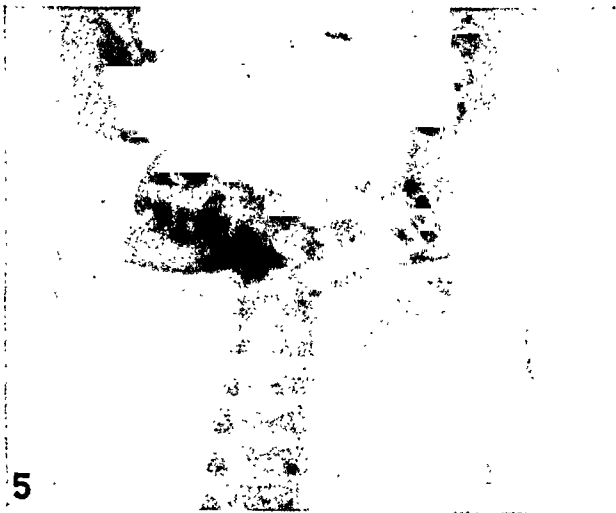


PLATE 139

- FIG. 5. Case 1. Anteroposterior roentgenogram, showing forking of vertebral column in mid-thoracic region. The effective length of the vertebral column is reduced by strong lateral curvature of the two halves.
- FIG. 6. Case 1. Roentgenogram of lateral half of fetus, showing the extent of cervical and thoracic vertebrae. The strong lordosis has brought the spinous processes of the divided thoracic vertebrae close to the base of the skull.
- FIG. 7. Case 2. Photograph after sectioning in the sagittal plane. There are many features similar to those of case 1 (Fig. 2): absence of neck; very large foramen magnum; divided cervical vertebrae; displacement of abdominal viscera (liver, large intestine and stomach) into the thorax through a large opening in left side of diaphragm. There is a very large meningocele covered with skin, arising by a narrow passage from the thin roof of the cervical spinal cord immediately opposite the divided vertebrae.
- FIG. 8. Case 2. Anteroposterior roentgenogram showing division of cervical vertebrae. The irregular longitudinal cleft in the photograph is an artefact. The photograph was taken after the fetus had been bisected and the two halves laid together again.
- FIG. 9. Case 2. Lateral roentgenogram of base of skull and upper vertebrae, showing lordosis of the divided vertebral column. There is a very large foramen magnum.



Dodds

Anterior and Posterior Rhachischisis

EMBOLI OF BRAIN TISSUE IN FETAL LUNGS*

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There are but two reports in the literature of fetal pathology of embolism and growth of nervous tissue in the lungs. For this reason, another case of this condition will be described and discussed in this paper.

REPORT OF CASE

Necropsy was done upon the body of a white female infant, 2 days old, measuring 55 cm. and weighing 2600 gm. by Dr. Mary Zeldes at the Department of Pathology of the Cook County Hospital (No. 277-1939). The anatomical diagnosis was: Anencephaly with meningoceles herniating through defects in the frontal bone; congenital cysts of all pulmonary lobes; fatty degeneration and passive congestion of the liver, and passive congestion of the spleen and intestine.

Only the findings related to the subject of this report will be described in detail. The skull was very small. The suture lines were irregular. In the area immediately above the bridge of the nose there was herniation of a soft, cystic, red-purplish mass measuring 30 by 28 by 15 mm. Adjoining this and projecting over the inner canthus of the left eye was another mass measuring 14 by 11 by 7 mm. and of similar consistency and color. The lungs were subcrepitant. Their surfaces were studded with light pink-tan, elevated areas up to 6 mm. in diameter, which collapsed when punctured. On section, the lungs appeared purple-red, with lighter areas similar to the ones on the surface. They were slightly moist with a frothy, bloody fluid. Both pleural cavities were free. The diaphragm was at the third interspace on the left, and at the third rib on the right side.

The necropsy unfortunately was performed under circumstances which gave no opportunity to preserve external parts for later investigation. While the examination of the head was in progress, I was impressed by the peculiar configuration of the skull. It was not that of typical anencephaly; on the contrary, the cranial cavity was, with the exception of the small openings in the frontal bone, closed by bone which was heavier even than

* Received for publication February 28, 1941.

normal. The calvarium was several millimeters thick and no fontanelles were present. The skull was covered by scalp with normal hair. In the cranial cavity, an essentially normal brain stem was found, but there were no definite hemispheres. The meninges continued through the defects in the frontal bone into the two protrusions seen externally on the forehead.

Microscopic examination of the lungs (Figs. 1 and 2) revealed that the foci, referred to as cysts in the gross diagnosis, consisted of an extremely loose tissue in which connective tissue fibrils could not be demonstrated even with an azan stain or silver impregnation. Only from the borders and from structures running through the foci did fibers penetrate into them for a short distance. Cells were scarce in these areas except in the vicinity of other tissues bounding or traversing them. Staining with phosphotungstic acid-hematoxylin revealed fibers not demonstrable with connective tissue stains; they could, therefore, be regarded as glial fibers. This, together with the general structure of the tissue, was evidence that the foci consisted of central nervous tissue, mainly glia. They were sharply separated from the surrounding normal lung tissue and from the pleura by a layer of connective tissue similar to that separating lobules of the lung from each other and from the pleura. Groups of small cavities like compressed alveoli could be seen in a few places at the border of the foci. Ducts lined with high columnar epithelium, and very wide, thin-walled blood vessels, penetrated the foci and divided there into smaller branches (Fig. 2). They were surrounded by scanty connective tissue; no muscle, cartilage, or glands were found in their walls or elsewhere within the foci. No indications of inflammation were seen either in these areas or in the lung tissue surrounding them. The lungs were only partly inflated, in accordance with the short duration of extra-uterine life.

DISCUSSION

The two reports in the literature in which similar disseminated foci of nervous tissue in the lungs are described also record severe malformations of the brain. This is true, also, of two cases* recently observed by Dr. Edith L. Potter at the Chicago Lying-in Hospital. Askanazy¹ was the first to describe this condition in

* To be published. I am indebted to Edith L. Potter for her kindness in showing me sections of the lungs of her cases.

FOREIGN BODY GIANT CELL GRANULOMA OF THE SPINAL CORD ASSOCIATED WITH SPINA BIFIDA *

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The case reported in this paper affords a natural reproduction of an experiment previously reported by Hassin.¹ The introduction of a foreign body into any portion of the central nervous system is necessarily associated with the rupture of capillary blood vessels, and the resultant reactive phenomena are, therefore, modified by the admixture of hematogenous elements. In the present case the cellular reaction about fragments of hair located within the substance of the spinal cord was of special interest for the foreign material had entered the nervous system accidentally through an arhaphic defect in the spinal cord and its meninges. There was, in a newborn infant, an ulcerated lumbosacral spina bifida, with exposure of the spinal cord and drainage of spinal fluid. The skin below the cutaneous defect showed hypertrichosis and some fragments of hair had entered the spinal cord, producing a characteristic cellular reaction.

REPORT OF CASE

History. A white female, 33 days of age, born 2 weeks prematurely on May 20, 1939, was admitted to the Cook County Hospital because of the drainage of spinal fluid from a spina bifida in the lumbosacral region.

Examination. The infant was poorly nourished. The essential finding was a mass, 2.5 by 2.5 by 4 cm. in diameter, in the lower lumbar region, surrounded by edematous tissue with spinal fluid escaping through a central defect. The mass bulged when the infant cried and the edges were necrotic.

Course. Ventricular puncture yielded an opalescent fluid under increased pressure. The Pandy reaction was 4 plus and there were 110 cells per cmm. There were 10 mg. of sugar and 600 mg. of chlorides per 100 cc. The culture of the spinal fluid revealed *Staphylococcus aureus*. The child did poorly, developed meningitis and died on July 14, 1939.

Necropsy

The anatomical diagnosis was spina bifida lumbosacralis; marked internal hydrocephalus; cloudy swelling of the kidneys, myocardium and liver; septic softening of the spleen.

* Read before the Chicago Pathological Society, October, 1940.

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1908. His case differs from the one reported here in the greater growth-intensity of the dislocated nervous tissue, invading lymph vessels of the lung and even a lymph gland. Askanazy considered his case to be one of multiple primary tumors which were not typically malignant. In Hückel's² case, a child with a frontal meningocele and comparatively little involvement of the brain lived 4 years. In its lungs, several foci much like those of the present case were found. The nervous tissue had grown into pulmonary alveoli without affecting their walls or any part of the lung. Hückel, therefore, did not regard the foci as tumors. It is possible that in his case the respiratory movements favored the extension of the dystopic tissue into the alveoli.

In the present case, there is not the slightest evidence of tumor-like behavior of the nervous tissue. Its extreme scarcity of cells also indicates a slow rate of growth in the stage observed. It cannot be denied, however, that the tissue, removed from its normal site and not being part of a harmoniously developing organ, may, soon after its deposition in the lungs, have multiplied to a greater extent than under normal circumstances.

The constant coincidence of foci of brain tissue in the lungs with severe changes in the brain points to a close connection between the two as has been assumed by previous observers. A detailed account of the changes in the head may, therefore, be essential for an explanation of the findings in the lungs. In the case here reported, the configuration of the head, including the openings in the frontal bone with meninges herniated through them, gave the impression of being a sequel of a severe injury rather than a malformation caused by intrinsic factors. The latter alternative can be ruled out because no deviation in a developmental process could possibly cause such bilateral openings in the skull and meninges as were found here.

In many human embryos of the late first or second month, as seen following abortions, the brain, which still consists of thin-walled cavities, is ruptured after it had been normally closed and the primordia of meninges and skull formed. In some the neural tube is torn along the line of closure in its dorsal wall. Also openings with protrusion of brain substance can be found in various, irregularly located points. Although it is safe to assume that almost all of these findings in embryos are postmortem artefacts,

There was separation of the cranial bones with enlargement of the skull. Below the cutaneous defect in the lumbosacral region, there was a moderate growth of hair. The cerebral ventricles were dilated, the ependyma was smooth and the cerebral cortex greatly reduced in thickness.

Microscopic Examination

Above the cutaneous defect the spinal cord presented the arhaphic variety of dysrhaphic malformation as described previously by Lichtenstein.² It consisted of a flat plate of spinal cord substance lined on its ventral aspect by a greatly thickened and fibrotic pia mater and having in its midportion a rudimentary anterior median fissure containing many blood vessels. Laterally this medullary plate gave rise to large bundles of nerve roots which deviated laterally and ventrally, many of them entering spinal ganglia. The dorsal portion of this medullary plate was composed of gray spinal cord substance rich in capillary blood vessels, the latter merging gradually into a densely fibrotic connective tissue layer which extended into the subcutis. The ventral white columns appeared normal except laterally where there was a moderate increase in fibrillar reticulum. At a more rostral level (Fig. 1) the arhaphic defect was no longer present, the spinal cord being closed dorsally and completely enveloped by leptomeninges.

Under low magnification the dorsal columns were seen to be the site of a spherical mass which completely replaced the spinal cord parenchyma in this region. This mass was encapsulated, sharply separated from the surrounding tissue, and its central portion as well as its capsule were rich in giant cells harboring spherical masses of amorphous material. In some microscopic fields this amorphous material seen in longitudinal section was easily recognized as fragments of hair (Fig. 2). The large cells were of the foreign body giant cell variety consisting of irregular protoplasmic masses containing a variable number of small, round, chromatin-rich nuclei and in some cells as many as twenty-five nuclei were present (Fig. 3). The surfaces of the segments of hair were plastered with innumerable giant cells and in transverse section the hairs appeared to be completely enclosed by them. Where the segments of hair were fine, two por-

they still show that in this period the comparatively thin wall of the ventricles, consisting of the brain and of the primordia of meninges, skull and skin, is, mechanically, a *punctum minoris resistentiae*. The immediate impression upon examining the head of the infant described here, was that it had suffered and survived a rupture of this sort. The findings in the lungs are best explained by the assumption of a blunt injury to the brain, followed by embolism of nervous tissue in the lungs.

Two possibilities of transportation of crushed brain substance to the lungs are to be considered: embolism and aspiration. We can be practically certain that the former happened in this case. Aspiration would require not only respiratory movements, but also a highly developed lung parenchyma. Without alveoli that can expand to many times their collapsed condition, even respiratory movements such as may occur during temporary anoxemia of the embryo could never succeed in bringing particles to the periphery of the lungs immediately underneath the pleura. The findings in the present case give evidence of the incomplete development of the lung parenchyma at the time of the embolism: There was conspicuous growth of the foci with but slight compression of lung tissue, and bronchi grew and branched after the brain tissue had reached the lungs. The shape of the foci and of the bronchi in them and the absence of compressed lung parenchyma around the bronchi are in favor of growth of the bronchi into the brain tissue rather than enlargement of the foci around bronchi already surrounded by alveoli. The vascular network of the lungs, on the other hand, must have been quite well developed at the time of the embolism. Only this can explain the presence of so many scattered and well isolated foci. We may, therefore, limit the time at which the anomaly in question originated to a period after the establishment of a complex system of capillaries in the lungs and before the formation of the definitive lung tissue. This coincides with the previously mentioned period of great vulnerability of the brain. Consequently, we may conclude that, during the second month of pregnancy, trauma caused the skull to rupture in the frontal region, destroyed part of the brain and at the same time forced particles of brain into the circulation. In later stages the brain and its coverings are comparatively much stronger and an injury leading to their rupture could scarcely be survived.

tions might be enveloped by a single giant cell, whereas many of the wider strands of hair were only partially enveloped. The central portion of the nodule contained large numbers of distended, fat-laden histiocytes enclosed in a meshwork of reticulum fibrils. The peripheral portions consisted of a dense connective tissue capsule rich in collagen fibers staining deep red by van Gieson's method. Scattered throughout were many giant cells surrounding segments of hair and many naked particles of hair embedded in a dense layer of connective tissue. This nodule was separated from the surrounding spinal cord substance by a narrow zone of fibrillar glia. The spinal cord presented no other changes of significance.

DISCUSSION

From the microscopic findings it is evident that we are dealing with a foreign body giant cell granuloma of the spinal cord, the foreign elements being fragments of hair which had entered the substance of the spinal cord through a defect in the overlying meninges, vertebral neural arch and cutaneous structures. Such defects may be arhaphic in nature, that is, they may result from nonclosure of the primitive neural groove; or they may be dysrhapic, resulting from defective closure. The fusion defects in such conditions are not restricted to the neural structures but involve the meninges, vertebrae and overlying skin as well. In the case described the spinal cord at one point had developed from the unclosed neural groove. It belongs, therefore, to the arhaphic variety of myelodysplasia. In some instances, as it was in our case, the skin about the cutaneous defect showed hypertrichosis. Some fragments of hair-shafts had entered the spinal cord through the defect in its overlying structures and had penetrated into segments of the cord beyond the site of the arhaphic defect, provoking a characteristic reaction.

The histopathologic changes produced in the spinal cord by the presence of hair were similar to those produced experimentally by the insertion of pledgets of cotton into the cerebral parenchyma of dogs by Oldberg and studied microscopically by Hassin.¹ In these experiments the types of cells present were dependent upon the duration of the experiment. Early, that is, in animals that died from 1 to 8 days after the operation, the reacting cells were

As was mentioned before, the relations of the nervous tissue to the bronchi and the surrounding lung parenchyma make it highly probable that the bronchi grew into the brain tissue. They branched in the abnormal surroundings, but did not form alveoli or other respiratory surfaces. Likewise no cartilage, muscle or glands were developed in their walls. This may be an indication that normally an influence of, or an interaction with, normal amounts of mesenchyme has to occur in order to enable full development of bronchial walls and formation of respiratory structures. The brain tissue grew, according to the pictures seen, in a benign manner and was indifferent to the surrounding lung tissue.

Both Askanazy and Hückel favored the assumption of embolism against aspiration, for reasons similar to those given. In all cases, including those observed by Potter, dystopic nervous tissue was found in the lungs exclusively. Fetal circulation, however, unlike that of the adult, must have deposited brain particles in other organs too, owing to the communication between pulmonary artery and aorta established by the ductus arteriosus. It seems that conditions in the lungs are more favorable for growth of such emboli than in any other organ. It cannot be determined what property of the embryonic lung tissue accounts for this peculiarity.

The findings in the skull of this case illustrate the moulding influence of the brain upon its bony capsule. The skull, primarily formed to cover a normal brain, was not exposed to the pressure of growing hemispheres after the injury. As a consequence, the bones reached each other earlier, leaving no fontanelles between them. It cannot be decided whether their abnormal thickness is due to the distribution of the normal amount of bone over an abnormally small surface, or to a diminution of normal bone resorption because of reduced intracranial pressure.

SUMMARY

Multiple foci of brain tissue were found in the lungs of a newborn infant with frontal meningoceles and a severe defect of the brain. As is evident from four reports of very similar cases gathered from the literature and personal communications, such foci in the lungs occur only in association with rather severe disturbances in the structure of brain and skull.

It is considered most probable that during the second month

chiefly lymphocytes, plasma cells, macrophages and fibroblasts and no giant cells or gitter cells were seen. In a dog that died after 12 days, fibroblasts and foreign body giant cells were numerous whereas lymphocytes and plasma cells were rare. The microscopic findings in our case were similar to those in the latter experiment. As in Hassin's case, the foreign bodies were plastered by large protoplasmic giant cells or surrounded by fibroblasts. It was readily apparent that the foreign body giant cells could not ingest the particles of hair because of their large size and chemical nature. With the development of fibrocytes and the formation of dense masses of collagenous connective tissue, many of the giant cells had disappeared, leaving the naked hair enveloped in a connective tissue scar. The maturity of the connective tissue response was such as to establish a strong presumption that inclusion of these hairs had occurred before birth and that the resulting granuloma was actually congenital.

Foreign body giant cells developing in the central nervous system are no different from those forming elsewhere in the body. They are undoubtedly mesenchymal in origin and in no way related to the macroglia. When the nervous parenchyma is invaded by smaller foreign elements, as for example the larvae of *Trichinella spiralis*, the reacting cells, according to Hassin and Diamond,⁸ are rich in cytoplasm and exclusively glial in nature. According to these authors the majority of these cytoplasmic glial cells fuse, forming syncytia, but in many places glial nodules develop which enclose the trichina larvae. The changes seen in our case were similar to those found when suture material is introduced into the central nervous system.

Localized areas of hypertrichosis over the spine are strongly suggestive of an underlying spina bifida occulta. Pilonidal cysts, dermoid cysts and teratoid tumors are also frequently found. It is important to note, however, that in our case the segments of hair had entered the spinal cord directly and that no evidence of a dermoid tumor was found.

SUMMARY

The microscopic changes produced by the entrance of hair into the substance of the spinal cord are described and the pathogenesis of the lesion resulting from spina bifida is discussed. The ma-

of embryonic life a blunt injury caused the brain to rupture and that this was followed by embolism of multiple particles of nervous tissue in the lungs. No evidence of tumor-like growth of the foci in the lungs could be obtained.

NOTE: I wish to acknowledge the kindness of Walter Schiller, Director of Laboratories of the Cook County Hospital, in permitting me to describe this case.

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DESCRIPTION OF PLATE

PLATE 141

FIG. 1. Photomicrograph of one of the foci of nervous tissue in the lungs. Azan stain. $\times 18$.

FIG. 2. Part of the same section shown in Figure 1, showing bronchi lined with high columnar epithelium, and congested thin-walled blood vessels in a loose glial stroma with few cells. $\times 100$.

24 to 48 hours have elapsed. Symptoms due to infection need not be confused with hypoglycemic symptoms since the latter appear in 1 to 5 hours after the injection of insulin. When infection occurs, the rabbit becomes less active, shows no desire for food, the eyelids droop, and in general the animal looks ill. Respirations may be increased. Fibrillary twitchings or coarse tremors may be present. Tremors may be more evident when the rabbit is grasped and held in the air. Some rabbits give a sensation of limpness and do not struggle when lifted off the table. The temperature may rise to 104° F. after 24 to 48 hours and return to normal after 72 hours. Paralysis was noted twice among eleven rabbits. One rabbit had weakness of all extremities after 24 hours with complete paralysis after 44 hours. A second rabbit, which was injected intracerebrally with a virus suspension from an infected rabbit, developed paresis of the extensor muscles of the head and neck on the second day and died on the third day. In some rabbits frank signs of infection are lacking although lesions will be found on histological examination. Close inspection may reveal some awkwardness in gait or unsteadiness after a few days.

DISCUSSION

The disease in the rabbit differs, obviously, in many respects from the disease in the monkey. The incubation period and clinical course are shorter. That these differences exist need not be at all surprising if the significance of hypoglycemia is recognized, since the disturbance in carbohydrate metabolism as it exists in the monkey was not reproduced at all in the rabbit. In the monkey, according to my concept of the factor of susceptibility, the virus grows and invades only when the blood sugar falls low enough to permit growth and invasion. Such periods of hypoglycemia may occur several times during 24 hours. Resistance to infection and variability in incubation period in the monkey may thus be due, in part, to variations in the degree of the disturbance in carbohydrate metabolism.

In these experiments the virus is enabled to grow and invade only during the period of induced hypoglycemia. As the effect of the insulin wears off and the blood sugar rises to normal levels, the virus is apparently prevented from growing and invading with

the rapidity reached during hypoglycemia. Those neurons harboring an overwhelming dose of virus will be unable to destroy it and will eventually die because intracellular metabolic processes will be disrupted. Death of the animal results from destruction of neurons in vital medullary centers. Death occurring as soon as 14 hours after inoculation may be attributed to severe and prolonged hypoglycemia, to a potent virus, and to marked involvement of medullary centers. If the disturbance in carbohydrate metabolism as it exists in the monkey were reproduced in the rabbit, the lesions and clinical course would probably be duplicated.

SUMMARY

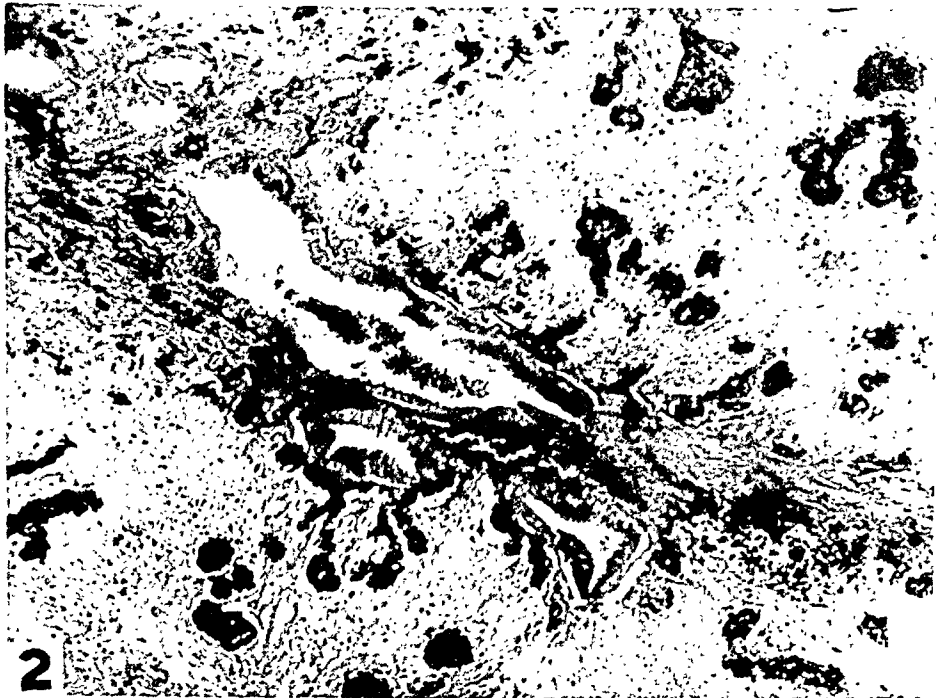
1. It is suggested that disturbance in carbohydrate metabolism, especially hypoglycemia, may be an important factor in determining susceptibility to the virus of poliomyelitis, both in man and in the monkey. Hypoglycemia reduces cellular oxidations, causing a cellular asphyxia of mild, moderate, or severe degree depending on the degree of hypoglycemia. That this asphyxia lowers the resistance of the individual cell and of the organism in general to invasion by the virus may be the mechanism of increased susceptibility.

2. The blood sugar of the rhesus monkey during tolerance tests has been observed to fall as low as 60 mg. per 100 cc. It is suggested that this hypoglycemia is responsible for the susceptibility of the rhesus monkey to the virus of poliomyelitis.

3. The blood sugar of the fasting rabbit is maintained at or above 100 mg. per 100 cc. By depressing the blood sugar to 60 mg. or less, it has been possible to produce neuronal injury in the anterior horns with the virus of poliomyelitis, both by intracerebral and by intranasal inoculation. Suspensions prepared from the spinal cords of infected rabbits, when injected into monkeys by the intracerebral route, cause primary neuronal injury and necrosis along with inflammatory changes.

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† Abstract of paper presented at the meeting of The American Society for Experimental Pathology held at Chicago, Illinois, April 17, 18 and 19, 1941.

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- Carcinoma of ceruminous gland.** (*Warren and Gates*: July, 640*; November) 821
- Carcinoma of the parathyroid gland.** (*Meyer and Ragins*: July) 637*
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- Cartilage**—A quantitative study of reversible stabilization of polysaccharide in dyed . . . (*Hass*: July) 589*
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- Cathepsins**—The behavior of cellular proteinases (. . .) in experimental tuberculosis of rabbits. (*Weiss*: May) 468†
- Cavities in the silicotic lung.** A pathological study with clinical correlation. (*Vorwald*: September) 709
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- Central nervous system irritation following injection of testicle extracts.** (*Winternitz, Mylon and Katzenstein*: July) 642*
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- Cerebral arteries**—Structure of the small . . . in hypertension. (*Baker*: January) 39
- Certain physiological differences between shock and hemorrhage.** (*Morgan, Lieber and McGrew*: July) 604*
- Certain specific and immunopathologic features of tuberculosis.** (*Corper*: September) 681
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- Observations on the infection of . . . with *B. tularensis*, *Brucella* and *P. pestis*. (*Buddingh and Womack*: May) 441†
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Neural and humoral responses from hypothalamic stimulation in cat and monkey. (<i>Bender and Weinstein</i> : May)	438†
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Oral administration of carcinogen — Experimental squamous cell carcinoma of the forestomach in mice and the method of induction by... (<i>Lorenz and Stewart</i> : May)	455†
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- Circumstances and postmortem findings, especially skin lesions, in accidental electrocution. (*Helpern and Strassmann*: July) . 592*
- Cirrhosis—Effects of dietary yeast on developing and healing carbon tetrachloride . . . in the rat. (*Post, Earle and Victor*: May) . 460†
- Coagulation time of the blood and mural vascular lesions as determinants of thrombosis. (*Katzenstein, Winternitz and Mylon*: July) 594*
- Coccidioidomycosis—A study of latent lesions of . . . correlated with coccidioidin skin tests. (*Butt and Hoffman*: July) 579*
- Comparative experimental studies of 200 kilovolt and 1000 kilovolt roentgen rays. III. The biologic effect on the skin of the albino rat. (*Gall, Lingley and Hilcken*: May) 319
- Comparison of hemoglobin production in experimental anemia of severe and moderate degree. (*Robscheit-Robbins*: May) 462†
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- Congenital nodular glycogenic degeneration of the myocardium. (*Olsen and Cooper*: January) 125
- Corpus luteum—The antigenic relationship of alcohol-soluble substances of . . . to those of testis and brain. (*Lewis*: September) . 725
- Correlation between anatomical changes and the allergic state in tuberculous guinea pigs. (*Woodruff, Willis and Kelly*: July) . . 575*
- Corynebacterium diphtheriae*—Infection of normal and passively immunized chick embryos with . . . (*Cromartie*: May) 411
- Cystic disease of the lung—Epithelial metaplasia in congenital . . . Its possible relation to carcinoma of the bronchus. (*Womack and Graham*: September) 645
- Cystic fibrosis of pancreas. (*Erb*: July) 634*
- Cystine—Influence of diet on liver lesions caused by excess dietary . . . (*Earle and Victor*: May) 444†
- Cytologic response of rats and mice to a strain of greening streptococci. (*Gross, Cooper and Phillips*: May) 377
- Degenerate versus multipolar neurons in sensory ganglia. (*Truex*: March) 211
- Degeneration of the adrenal cortex produced by germanin. (*Humphreys and Donaldson*: September) 767
- Description of specimens of pellagra. (*Moore, Spies, Cooper and Goldblatt*: July) 585*
- Detection of mild or subclinical scurvy. (*Rinehart*: May) 461†
- Development of the agent of lymphogranuloma venereum in the yolk sac of the chicken embryo. (*Rake, Jones and Shaffer*: May) 460†
- Diabetes—Histological study of trophic effects of diabetogenic anterior pituitary extracts and their relation to the pathogenesis of . . . (*Ham and Haist*: November) 787
- On the mechanism of enhanced . . . with inflammation. (*Menkin*: May) 458†
- The neuro-insular complex of the pancreas: its possible rôle in . . . (*Simard*: July) 590*
- Dibenzanthracene—Effect of . . . on transplantable mammary adenofibroma of the white rat. (*Davis, Murphy and Emge*: January) 93

- Osteo-arthropathy**—Hypertrophic pulmonary... A pathologic study of six cases. (*Gall and Bennett*: July) 600*
- Pulmonary artery to left auricle anastomosis with hypertrophic... (*Mendlowitz and Leslie*: May) 458†
- Oxyuriasis**—Appendiceal... Its incidence and relationship to appendicitis. (*Ashburn*: November) 841
- Pancreas**—Cystic fibrosis of... (*Erb*: July) 634*
- Observations with differential stains on human islets of Langerhans. (*Gomori*: May) 395
- The neuro-insular complex of the...: its possible rôle in diabetes. (*Simard*: July) 590*
- The relation of the ... to the celiac syndrome. (*Farber and Maddock*: May) 445†
- Pantothenic acid deficiency**—Pathological changes in the mouse due to... (*Lippincott and Morris*: July) 588*
- Parasympathetic effects of phospholipids and erythrocytes.** (*Shwartzman, Bender and Wachtel*: May) 465†
- Parathyroid gland**—Carcinoma of the... (*Meyer and Ragins*: July) 637*
- Parathyroids**—Effects of long-continued ingestion of sodium phosphate upon the..., kidneys and bones of mature rats. (*Saxton and Ellis*: July) 590*
- Pathogenesis and pathology of experimental type I pneumococcus pneumonia in the monkey.** (*Loosli*: May) 454†
- Pathogenesis of arterial atrophy.** (*Moritz*: July) 597*
- Pathogenesis of intestinal radiation lesions.** (*Friedman and Warren*: May) 446†
- Pathological changes in the mouse due to pantothenic acid deficiency.** (*Lippincott and Morris*: July) 588*
- Pathology of arthritis deformans.** (*Goldberg*: July) 598*
- Pathology of the joint lesions in patients with psoriasis and chronic arthritis.** (*Bennett, Zeller and Bauer*: July) 599*
- Pathology of shielded arc welding.** (*von Haam and Groom*: July) 591*
- Pellagra**—Description of specimens of... (*Moore, Spies, Cooper and Goldblatt*: July) 585*
- Peptic ulcer**—Gastric acidity after various types of operation for ulcer in man. (*Lamm and Wangensteen*: May) 454†
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- Peripheral vascular reactions in anaphylactic shock of the mouse.** (*McMaster*: May) 457†
- Pertussis**—Brain changes in... (*Dolgopol*: July) 633*
- Phage-antiphage reaction**—Quantitative aspects of the... (*Hershey and Bronfenbrenner*: May) 449†
- Phagocytic activity of the oligodendroglia and amphicytes in the brain, spinal cord, and semilunar ganglion of the mouse during inanition.** (*Andrew*: May) 421
- Phosphatase**—A histochemical study of the distribution of alkaline... in various normal and neoplastic tissues. (*Kabat and Furth*: May) 303

- Diethylstilboestrol—Endometrial response to...in radium-induced menopause. (*Grauer, Beall and Wilson*: January) 87
- Diffusion of some animal viruses. (*Bourdillon*: May) 440†
- Dimethylaminoazobenzene—The non-neoplastic hepatic changes in rats fed...with special reference to pigment deposits. (*Edwards and White*: May) 444†
- Distinct types of antibody in the blood of rabbits carrying the transplanted V₂ carcinoma. (*Kidd and Friedewald*: May) 453†
- Distribution of vitamin A in ovaries and ovarian tumors. (*Popper and Ragins*: July) 587*
- Disuse atrophy of renal arteries. (*Bell*: July) 614*
- Early cancer of the gastro-intestinal tract. (*MacCarty*: July) 630*
- Effect of atropine and pilocarpine upon the sphincter of Oddi in human subjects. (*Bergh*: May) 439†
- Effect of dibenzanthracene on transplantable mammary adenofibroma of the white rat. (*Davis, Murphy and Emge*: January) 93
- Effect of glycogen on growth of Walker 256 tumor in rats. (*Ball*: May) 438†
- Effect of hepatectomy, and abdominal evisceration with and without hepatectomy on the serum phosphatase of the dog. (*Maddock, Trimble, Jensen and Appleby*: May) 456†
- Effect of insulin and insulin plus thyroxin on the metastasis of the Brown-Pearce epithelioma. (*Ryer and Murlin*: May) 463†
- Effect of pancreatic achylia on vitamin K absorption and prothrombin time. (*Sproul and Sanders*: July) 587*
- Effect of the pituitary growth hormone on the epiphyseal disk of the tibia of the rat. (*Ray, Evans and Becks*: July) 509
- Effective renal blood flow, filtration rate and functional excretory mass in essential hypertension; diodrast and insulin clearances. (*Foa, Woods and Peet*: May) 446†
- Effects of the continued administration of sulfathiazole and sulfapyridine to monkeys. (*Climenko and Wright*: July) 633*
- Effects of dietary yeast on developing and healing carbon tetrachloride cirrhosis in the rat. (*Post, Earle and Victor*: May) 460†
- Effects of hypoproteinemia on blood pressure in normal and hypertensive dogs. (*Erickson*: May) 444†
- Effects of long-continued ingestion of sodium phosphate upon the parathyroids, kidneys and bones of mature rats. (*Saxton and Ellis*: July) 590*
- Effects of syngenesiotransplants and of extracts of the anterior lobe of the bovine hypophysis on the age changes in the long bones and joints of mice. (*Silberberg and Silberberg*: March) 189
- Electric shock: importance of path, distribution and density of current in determining symptoms and pathology. (*Alexander and Weeks*: July) 601*
- Electrocution—Circumstances and postmortem findings, especially skin lesions, in accidental...(*Helpern and Strassman*: July) 592*
- Electron micrography of purified viruses. (*Stanley and Anderson*: July) 576*

- The effect of hepatectomy, and abdominal evisceration with and without hepatectomy on the serum... of the dog. (*Maddock, Trimble, Jensen and Appleby*: May) 456†
- Phosphotungstic acid hematoxylin staining — A mordant preparing formaldehyde-fixed neuraxis tissue for... (*Mullen and McCarter*: March) 289
- Physiological and pharmacological behavior of the human appendix. (*Dennis*: May) 443†
- Pilocarpine — The effect of atropine and... upon the sphincter of Oddi in human subjects. (*Bergh*: May) 439†
- Pituitary — Effect of the... growth hormone on the epiphyseal disk of the tibia of the rat. (*Ray, Evans and Becks*: July) 509
- Pituitary extracts — Histological study of trophic effects of diabetogenic anterior... and their relation to the pathogenesis of diabetes. (*Ham and Haist*: November) 787
- Plasma — Studies on the intravenous administration of bovine... to man. (*Kremen, Hall, Koschnitzke, Stevens and Wangenstein*: May) 454†
- Plasma protein — Shock: ... building in emergencies as influenced by intravenous digests. (*Whipple*: July) 609*
- Pneumococcus — The pathogenesis and pathology of experimental type I... pneumonia in the monkey. (*Loosli*: May) 454†
- Pneumonia — Experimental staphylococcic... in rabbits. (*Gaspar*: July) 634*
- Poliomyelitis — Experimental... in guinea pigs. (*Jungeblut and Sanders*: May) 452†
- Studies on the relation of "neurotropic" streptococci to... and its virus. (*Rosenow*: May) 462†
- The isolation of "poliomyelitic" streptococci from the stool in acute epidemic... (*Rosenow*: July) 641*
- The production of neuronal injury and necrosis with the virus of... in rabbits during insulin hypoglycemia. (*Sandler*: January) 69
- Polyneuritis — Visceral lesions in infectious... (infectious neuritis, acute polyneuritis with facial diplegia, Guillain-Barré syndrome, Landry's paralysis). (*Sabin and Aring*: July) 469
- Pregnancy in dogs — Experimental hypertension and... (*Dawson, Cressman and Blalock*: January) 31
- Present incidence of tuberculous infection. (*Carnes*: July) 632*
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- Production of antibodies in the popliteal lymph node of the rabbit. (*Ehrlich and Harris*: July) 633*
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- Electron microscope**—The structure of bacteria as shown by the
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- Encephalitis**—Isolation of the virus of herpes simplex and the
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- The complement-fixation test in the diagnosis of some types of
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- Environmental factors influencing fever in pulmonary tubercu-
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- Eosinophilic granuloma**—The nature of "solitary or..." of bone.
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- Eperythrozoon**—"Interference" in mixed infections of bartonella
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- Epithelial metaplasia in congenital cystic disease of the lung. Its
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Graham*: September) 645
- Epithelioma of lip in catfish. I. Pathology of spontaneous tumors.
II. Growth of intracorneal transplants.** (*Lucké and Schlumberger*:
May) 455†
- Equine encephalomyelitis in man.** (*Adler*: May) 407
- Ergot**—Neurofibromatous tumors of the ears of rats produced by
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July) 624*
- Erythrocyte**—Relation of age to the susceptibility of the...to
hypotonic saline solution. (*Cruz*: May) 442†
- Erythrocytes**—Production of elongated (pencil)...in chronic
hemorrhage. (*Isaacs and Rosenman*: May) 451†
- Estrogen**—Occurrence of urinary tract calculi in inbred strain (C_3H)
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- Histogenesis of... (*Foote and Anderson*: July) 497
- Exogenous tumors of the thyroid gland.** (*Mayo and Schlicke*:
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- Promin**—The treatment of experimental tuberculosis with...(sodium salt of P.P' diamino-diphenyl-sulfone-N,N'-dextrose sulfonate). (*Feldman, Hinshaw and Moses*: July) 578*
- Prothrombin**—Effect of pancreatic achylia on vitamin K absorption and...time. (*Sproul and Sanders*: July) 587*
- Lymph... (*Walker and Brinkhous*: May) 468†
- Psoriasis**—The pathology of the joint lesions in patients with... and chronic arthritis. (*Bennett, Zeller and Bauer*: July) 599*
- Pulmonary artery to left auricle anastomosis** with hypertrophic osteoarthropathy. (*Mendlowitz and Leslie*: May) 458†
- Quantitative aspects of the phage-antiphage reaction.** (*Hershey and Bronfenbrenner*: May) 449†
- Quantitative study of reversible stabilization of polysaccharide** in dyed cartilage. (*Hass*: July) 589*
- Rabies**—A nonvirulent irradiated...vaccine. (*Webster and Casals*: July) 582*
- A study of chick-embryo-adapted...virus. (*Dawson*: March) 177
- Rare malignant tumor of the thyroid** with postmortem findings. (*Polayes*: July) 638*
- Rate of disappearance of the eastern equine encephalomyelitis virus** from the blood of rabbits following the injection of India ink. (*Kempff, Pierce and Soule*: May) 453†
- Red blood cell volume, total and circulating, as determined with radioactive iron.** (*Hahn*: May) 447†
- Relation of age to the susceptibility of the erythrocyte to hypotonic saline solution.** (*Cruz*: May) 442†
- Relation of the "myocardial reticulocyte" to the Aschoff nodule.** (*Clawson*: July) 597*
- Relation of the pancreas to the celiac syndrome.** (*Farber and Maddock*: May) 445†
- Relative susceptibility of the synaptic terminals and of the perikaryon to arrest of the circulation of the brain.** (*Kabat and Schadewald*: November) 833
- Renal arteries**—Disuse atrophy of... (*Bell*: July) 614*
- Renal biopsies from hypertensive patients.** (*Castleman, Smithwick and Palmer*: July) 617*
- Renal tumors**—Functional structures in... (*Schiller*: July) 622*
- Reticulum cell sarcoma of lymph nodes.** (*Warren and Picena*: May) 385
- Retinoblastoma**—The histogenesis of glioma retinae. Report of early case with review of literature. (*Ch'in*: November) 813
- Rhabdomyomatosis of the heart in a guinea pig.** (*Hueper*: January) 121
- Rhachischisis**—Anterior and posterior... (*Dodds*: November) 861
- Roentgen rays**—Comparative experimental studies of 200 kilovolt and 1000 kilovolt...III. The biologic effect on the skin of the albino rat. (*Gall, Lingley and Hilcken*: May) 319
- Pathogenesis of intestinal radiation lesions. (*Friedman and Warren*: May) 446†

- Experimental colloid droplets in renal epithelium. (*Johnson and Smetana*: July) 635*
- Experimental hypertension and pregnancy in dogs. (*Dawson, Cressman and Blalock*: January) 31
- Experimental measles. The lymphoid tissues of animals inoculated with the virus of human measles. (*Gordon and Knighton*: March) 165
- Experimental ocular tuberculosis in normal and immunized rabbits. (*Angevine and Huntington*: March) 155
- Experimental poliomyelitis in guinea pigs. (*Jungeblut and Sanders*: May) 452†
- Experimental squamous cell carcinoma of the forestomach in mice and the method of induction by oral administration of carcinogen. (*Lorenz and Stewart*: May) 455†
- Experimental staphylococcic pneumonia in rabbits. (*Gaspar*: July) 634*
- Explantation as an aid to the study of kidney pathology. (*Allen and Youland*: May) 437†
- Extracts of spleen or yeast — The treatment of spontaneous breast adenocarcinomas in mice with... (*Lewisohn, Leuchtenberger, Leuchtenberger and Laszlo*: March) 251
- Eye — Experimental ocular tuberculosis in normal and immunized rabbits. (*Angevine and Huntington*: March) 155
- Factors regulating the absorption of iron in dogs as measured with the radioactive isotope. (*Balfour*: May) 438†
- Fat embolism — Necrosis of the bone marrow with... in sickle cell anemia. (*Wade and Stevenson*: January) 47
- Fate of tubercle bacilli phagocytized *in vivo* and *in vitro* by mononuclears derived from normal and immunized rabbits. (*Lurie*: July) 636*
- Fatty changes in the glomeruli of the kidneys. (*Simonds and Lange*: May, 466†; September) 755
- Fibromyxosarcomas — Primary... of the heart and pulmonary artery. (*Haythorn, Ray and Wolff*: March) 261
- Foreign body giant cell granuloma of the spinal cord associated with spina bifida. (*Lichtenstein and Kirshbaum*: November) . 873
- Functional activity of smooth muscle tumors of the uterus. (*Bryan and Warren*: May) 441†
- Functional structures in renal tumors. (*Schiller*: July) 622*
- Fungi — The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic... (*Moore*: January) 103
- Further observations on a gastric secretory depressant factor in gastric juice. (*Brunschwig and Rasmussen*: May) 440†
- Gallstones — Variation in the composition of... simultaneously formed in the gallbladder. (*Phemister and Aronson*: September) 673
- Ganglia — Degenerate versus multipolar neurons in sensory... (*Truex*: March) 211
- Gastric acidity after various types of operation for ulcer in man. (*Lannin and Wangensteen*: May) 454†

- Role of potassium in the survival time after bilateral nephrectomy. (*Durlacher, Darrow and Winternitz*: July) 614*
- Scorbutus — The detection of mild or subclinical scurvy. (*Rinehart*: May) 461†
- Sex hormones and lymphomatosis of fowls. (*Marine and Rosen*: May) 456†
- Shock — Certain physiological differences between...and hemorrhage. (*Morgan, Lieber and McGrew*: July) 604*
- Electric...: importance of path, distribution and density in determining symptoms and pathology. (*Alexander and Weeks*: July) 601*
- Morphologic changes in experimental... (*Lieber and Morgan*: July) 607*
- : plasma protein building in emergencies as influenced by intravenous digests. (*Whipple*: July) 609*
- Specific therapeutic...—the Hugh Young reaction. (*MacNeal*: July) 600*
- Studies on the blood histamine in rabbits during hemorrhage,... produced by manipulation of the intestines, and following the subcutaneous injection of histamine. (*Rose and Browne*: July) 606*
- The leukocytic response in experimental... (*Curphey and Ponder*: July) 602*
- The treatment of...by the intravenous administration of non-hematogenous macromolecular substances. (*Hueper, Martin and Thompson*: July) 608*
- The vascular and cellular dynamics of... (*Moon*: July) 610*
- Sickle cell anemia — Necrosis of the bone marrow with fat embolism in... (*Wade and Stevenson*: January) 47
- Significance of glucose and non-glucose-reducing substances in post-mortem blood. (*Hill*: May) 449†
- Silicosis — Cavities in the silicotic lung. A pathological study with clinical correlation. (*Vorwald*: September) 709
- Skin — Histologic changes in the...of mice following radiation from mercury arc. (*Grady, Blum and Kirby-Smith*: May) 446†
- Soluble antigen in lymphogranuloma venereum. (*Shaffer and Rake*: May) 464†
- Some factors in the development, localization and reabsorption of experimental amyloidosis in the rabbit. (*Dick and Leiter*: September) 741
- Some pathological aspects of human malaria. (*Cannon*: July) 580*
- Specific lesions of the small intestines in congenital syphilis. Two additional cases. (*Pearson and Palik*: July) 637*
- Specific therapeutic shock—The Hugh Young reaction. (*MacNeal*: July) 600*
- Sphincter of Oddi — The effect of atropine and pilocarpine upon the...in human subjects. (*Bergh*: May) 439†
- Spina bifida — Foreign body giant cell granuloma of the spinal cord associated with... (*Lichtenstein and Kirshbaum*: November) 873
- Spread of tubercle bacilli by sputum, blood and lymph in pulmonary tuberculosis. (*Long and Faust*: September) 697

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DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Rabbit No. 1. First passage, intracerebral inoculation. Thoracic segment; anterior horn cells in varying degrees of necrosis. Note loss of Nissl bodies. The cytoplasm is undergoing dissolution and the cell borders have become frayed. Capillaries are dilated. All sections were stained with hematoxylin and eosin. $\times 390$.

FIG. 2. Rabbit No. 13. First passage, intracerebral inoculation. Thoracic segment; anterior horn cells in varying degree of necrosis. Nissl bodies stain poorly and are in process of dissolution. Note swelling of axons. Capillaries are dilated. $\times 370$.

- Stilbestrol**—Testicular tumors in mice injected with... (*Shimkin and Grady*: May) 465†
- Streptococci**—The cytologic response of rats and mice to a strain of greening... (*Gross, Cooper and Phillips*: May) 377
- Structure of bacteria** as shown by the electron microscope. (*Mudd, Anderson, Polevitsky and Morton*: July) 576*
- Structure of the small cerebral arteries** in hypertension. (*Baker*: January) 39
- Studies in melanuria**. (*Rothman*: May) 463†
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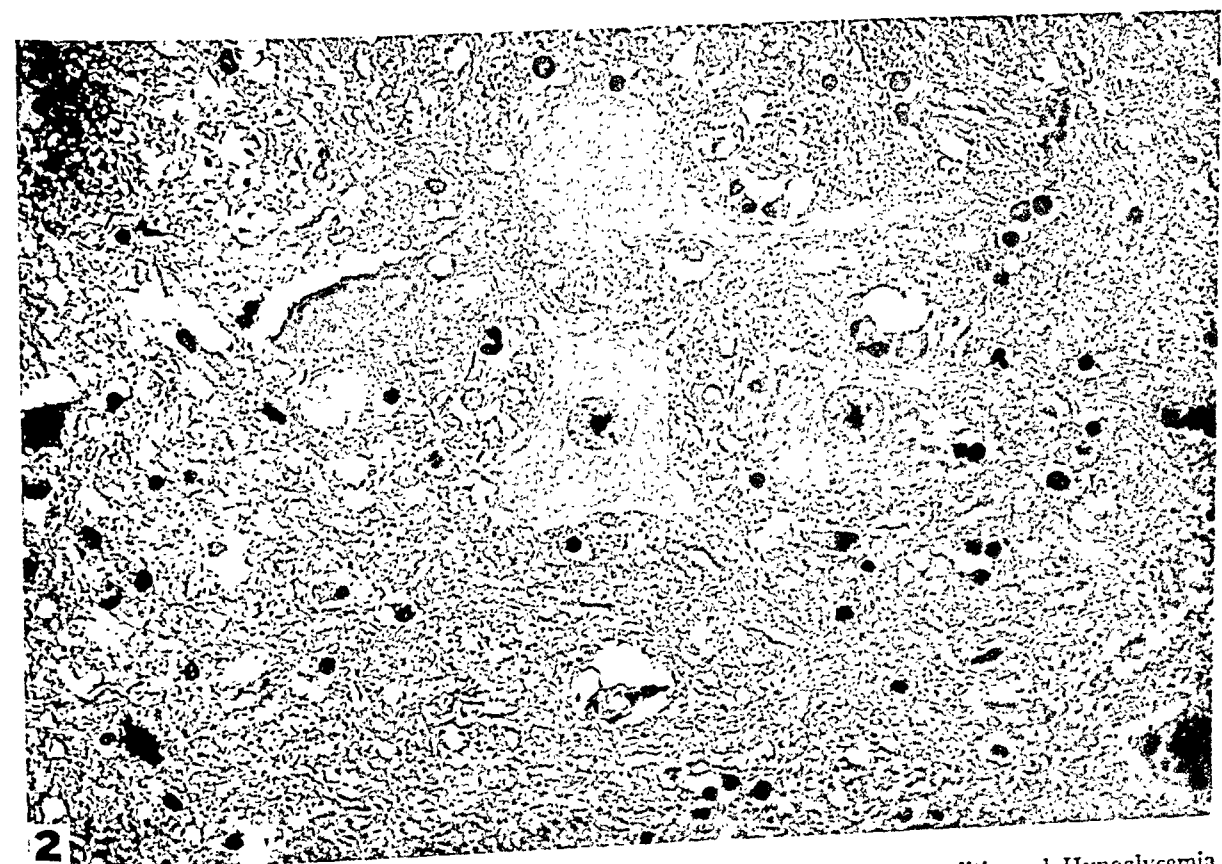
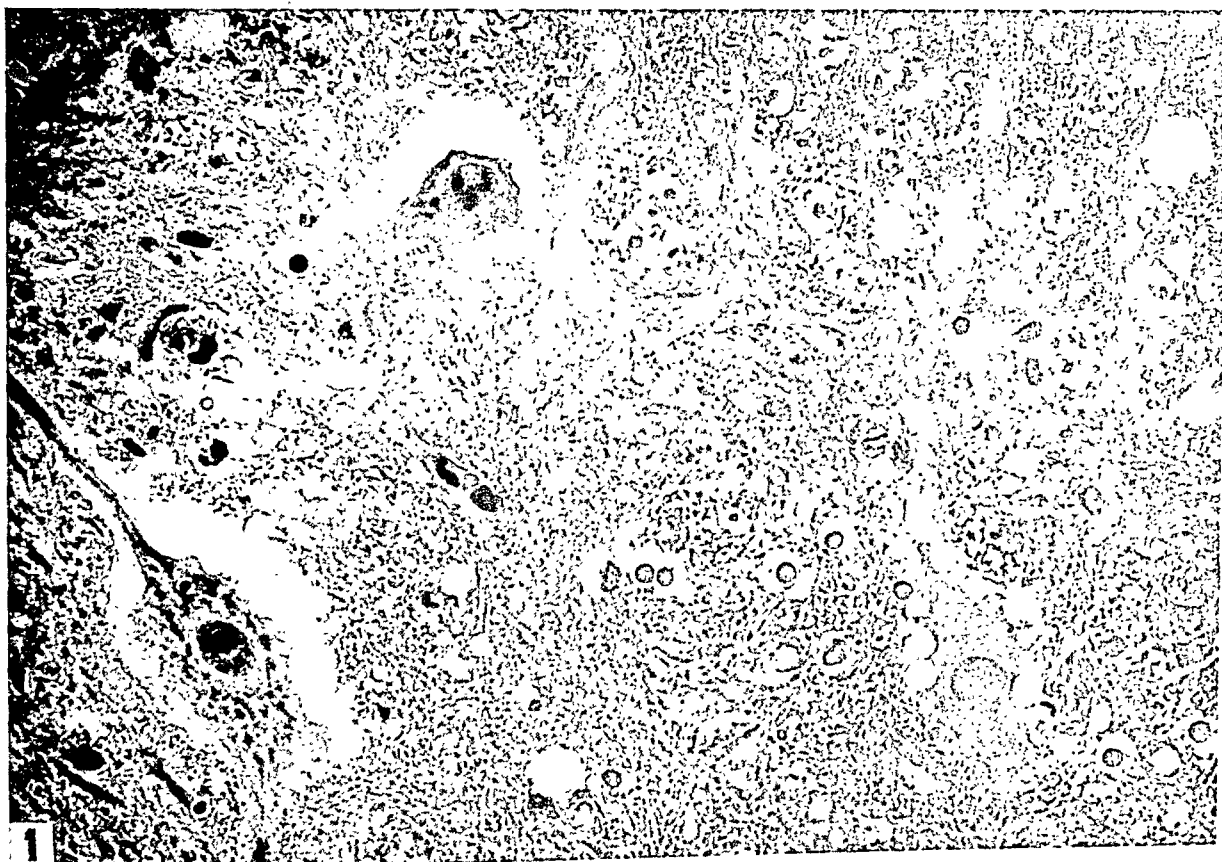
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PLATE 15

- FIG. 3. Rabbit No. 18. First passage, intranasal inoculation. Thoracic segment; moderate to severe necrosis of anterior horn cells. One neuron (marked by an arrow) is barely distinguishable from the ground substance. $\times 370$.
- FIG. 4. Rabbit No. 17. Third passage, intranasal inoculation. Motor neurons from medulla. Primary neuronal injury with loss of Nissl bodies. The pale areas take an eosinophilic stain with hematoxylin and eosin. $\times 360$.

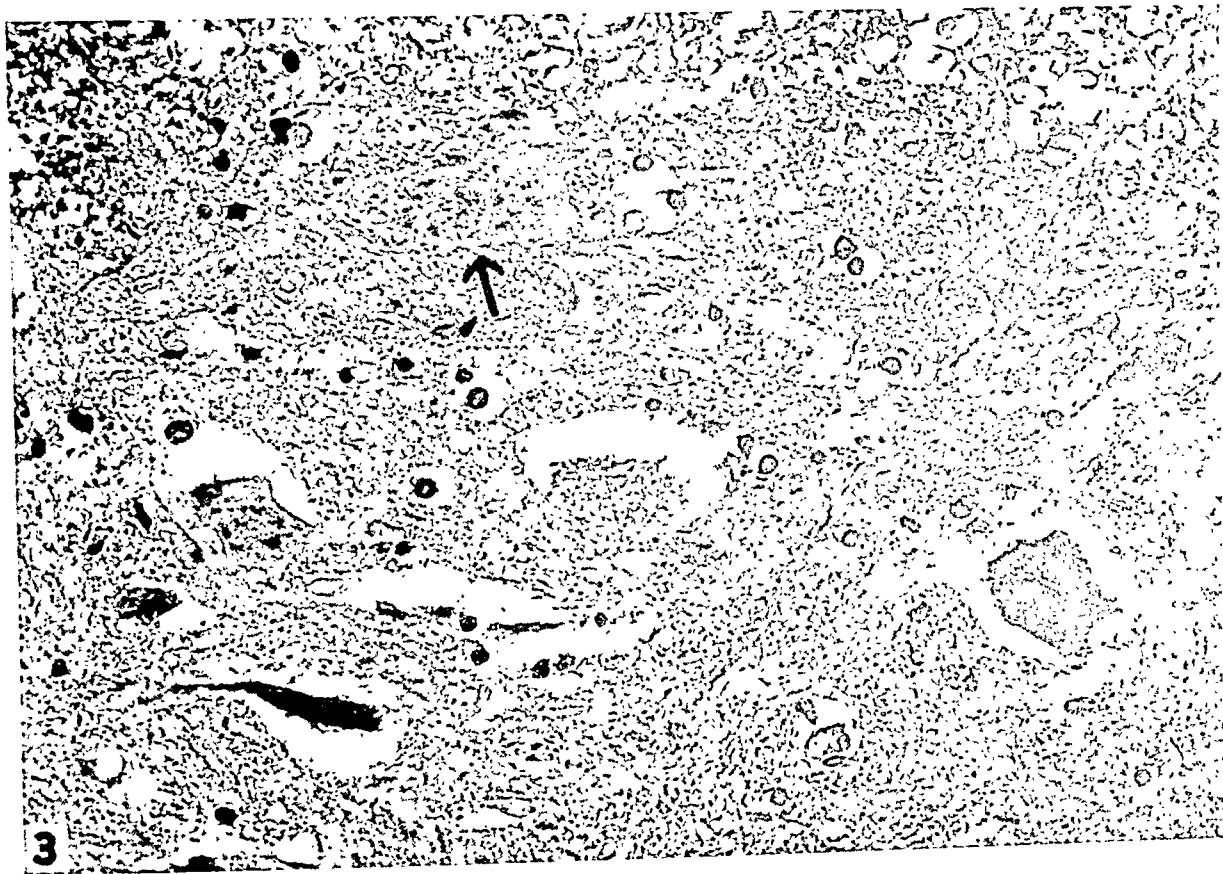


Poliomyelitis and Hypoglycemia

PLATE 16

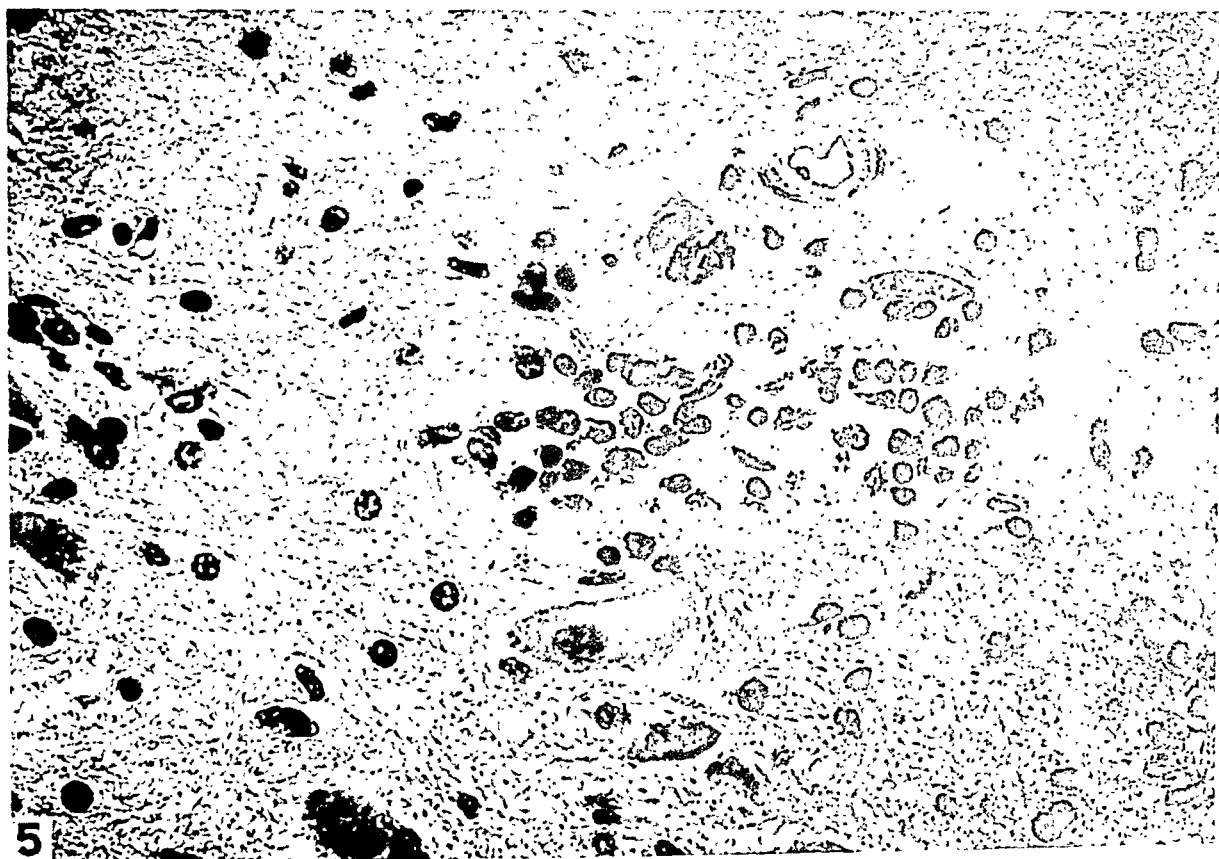
FIG. 5. Monkey No. 3. Thoracic segment; area of infiltration with polymorphonuclear leukocytes and small round cells. Severe neuronal necrosis and neuronophagia. $\times 650$.

FIG. 6. Monkey No. 4. Cervical segment; motor neurons from left anterior horn. Severe primary neuronal necrosis without interstitial infiltration. $\times 390$.



Poliomyelitis and Hypoglycemia

Sandler



Poliomyelitis and Hypoglycemia

TABLE I
Muscular Dystrophy in Biliary Fistula Dogs

Dog No.	Sex	Duration of biliary fistula	Muscle weakness	Muscle lesions	
				Necrotic lesions	Simple atrophy
1	F	6 months	None	+	0
2	F	7½	Slight	+++	Slight
3	F	7½	Slight	+	+
4	F	9½	Marked, unable to walk last 12 weeks	++	+++
5	M	11	Marked, unable to walk last week	++	+
6	F	11½	Marked, unable to walk last 2 weeks	+	+
7	F	12	Marked, unable to walk last 8 weeks	+	++
8	M	13	Slight	++	+
9	M	13½	Slight	++	+
10	M	14	Marked, unable to walk last 4 weeks	++	++
11	M	14½	Marked, unable to walk last week	++	++
12	F	26½	Slight	+	+
13	F	32	Marked, unable to walk last 2 weeks	++	+

Table I lists the animals in which a careful study of the dystrophic muscle changes was made. Ten of these animals received no bile or bile salts at any time during their course and the remaining three (dogs 2, 12, and 13) received none within the 6 months prior to study of the muscles. The dystrophy varied markedly in severity in this group. In dog 4, paresis was so extreme 6½ months after operation that the animal was able to walk only with difficulty. On the other hand, dog 12 was observed for over 2 years, during which time only moderate muscular weakness developed. This was insufficient to impair seriously the animal's activity. In dog 1 there were no overt symptoms in 6 months. However, in this animal as well as in all the others of this group, well developed muscular lesions were observed on microscopic examination.

The primary lesion of the skeletal muscles appeared to be a focal hyaline necrosis of a whole fiber or more commonly a short segment of it (Fig. 1). Necrosis was followed by phagocytosis and absorption of the necrotic sarcoplasm, and eventual collapse of the fibers. Replacement fibrosis was noted in advanced cases. In addition to the necrosis, there was variable and often marked proliferation of the subsarcolemmic nuclei, both about the sites of necrosis and in otherwise unaffected fibers. The nuclei were oval or elongated, at times centrally placed in the fiber, and occasionally in chains of six to ten. Little active regeneration of the destroyed portions of the fibers was observed, however.

MUSCULAR DYSTROPHY IN BILIARY FISTULA DOGS; POSSIBLE RELATIONSHIP TO VITAMIN E DEFICIENCY*

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Many of the chronic biliary fistula dogs which we have studied in our vitamin K experiments¹ developed marked muscular weakness, followed by atrophy of skeletal muscles. Examination of the affected muscles² revealed lesions similar to those of the nutritional muscular dystrophy which has been shown recently to be related to a vitamin E deficiency. Although our chronic biliary fistula dogs were maintained on an adequate diet, faulty absorption, incident to the absence of bile in the intestine, resulted in a deficiency of at least two of the fat-soluble vitamins; vitamin K, as indicated by a hemorrhagic tendency, and vitamin D, as indicated by the gradual development of osteomalacia. In view of the recent work on vitamin E and nutritional muscular dystrophy, it appears probable that an analogous deficiency of vitamin E was responsible for the muscular dystrophy in these animals.

MATERIALS AND RESULTS

The gallbladder-renal type of fistula was used.³ The animals were fed a diet consisting either of hospital scraps, of dog chow (Purina), or a standard mixed diet previously described.¹ Unoperated control dogs maintained on these same diets remained entirely normal and developed no muscle lesions. In the untreated biliary fistula animals, osteoporosis, duodenal ulcers, and intestinal disturbances, as described by Hawkins and Whipple,⁴ as well as the hemorrhagic diathesis due to hypoprothrombinemia,⁵ were frequent complications. In addition, it was noted that unless bile was fed by mouth, the animals regularly developed muscle lesions within 6 to 8 months after the fistula was established.

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were small and contained few or no germ cells. In the testes of dog 8 these changes were less extreme, and all stages of degeneration of the germinal epithelium, including extensive multinucleated giant cell formation (Fig. 2), similar to that described by Mason ⁶ in vitamin E deficiency in rats, were noted.

One animal, not listed in Table I, is of particular interest because of the effects of bile feeding. About the twelfth month after the establishment of the biliary fistula, moderate weakness and atrophy of the skeletal muscles were noted. These progressed gradually during the next 3 months, when the atrophy and paresis were both pronounced. At that time ox bile (75 to 100 cc. daily) was started by mouth and was continued during the last 6 months of this experiment. During this period there was considerable improvement of the muscle strength, but the muscular atrophy persisted. At autopsy, no necrotic muscle lesions could be found, although there was considerable proliferation of subsarcolemmic nuclei. Together with replacement fibrosis this suggested that parenchymatous degeneration had been present earlier. The progress of the dystrophy was arrested apparently by the bile feeding in this animal.

DISCUSSION

The lesions which we have observed in the chronic biliary fistula dogs were of essentially the same type as those described in the nutritional muscular dystrophy of rabbits and guinea pigs by Goettsch and Pappenheimer.⁷ Although the diets in their experiments were deficient in vitamin E, addition of wheat germ oil failed to prevent the development of the dystrophy. While Morgulis ⁸ found that vitamin E is essential for the prevention of the disorder in these animals, he believed that some other factor in addition to vitamin E, perhaps a part of the vitamin B complex, was concerned. Olcott ⁹ reported dystrophic muscle lesions in suckling rats of vitamin E-deficient mothers, similar to those described by Goettsch and Pappenheimer.⁷ Later reports have described the production of the dystrophy in adult rats.^{10,11} Knowlton, Hines and Brinkhous ¹² have shown that this nutritional dystrophy in rats can be prevented as well as cured by the subcutaneous administration of synthetic vitamin E (α -tocopherol acetate). Thus, from the data available, it is evident that muscu-

Simple atrophy of many of the muscle fibers unaffected by necrosis was present in some of the muscle groups, but this was a much less consistent finding than were the necrotizing lesions and the nuclear proliferation. In a very few instances in the markedly atrophic muscles, there was also a slight degree of adipose tissue replacement. Aside from the shrinkage in size and some pallor, the affected muscles were not abnormal to gross examination.

These degenerative, proliferative and atrophic changes were distributed in patchy fashion throughout the involved muscles. In this group of animals, no muscle groups were exempt, but those of the posterior extremities were affected most severely. This corresponded with the muscles which showed the most marked paresis during life. The patchy distribution of the lesions may account for the lack of any very exact correlation between the degree of paresis and the extent of the lesions in the sections studied. As a rule, only one section of each muscle group was taken. More extensive sampling might have resulted in a more exact correlation.

With the appearance of the atrophic muscle changes approximately 7 to 9 months after operation, many of the animals began to lose weight. In some this was 20 per cent or more of the original preoperative weight, although food consumption was adequate. The extent of the weight loss tended to parallel, in general, the degree of muscular atrophy.

The nervous system (brain, spinal cord and peripheral nerves) of seven of the animals of this group was studied carefully. Sections were stained both with hematoxylin and eosin and with stains for myelin sheaths. In addition stains for nerve endings (Ranvier) were studied in several of the dogs. No evidence of neuronal or glial changes, or of degeneration of tracts, peripheral nerves or motor end-plates could be found.

No lesions of cardiac muscle or of smooth muscle of the uterus, alimentary tract, bronchi, blood vessels or bile ducts were observed.

The testes of the males of this group of animals showed marked degenerative changes of the germinal epithelium, with impairment of or complete loss of spermatogenesis. In three of the animals these changes were extreme. Many of the seminiferous tubules

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DESCRIPTION OF PLATE

PLATE 17

FIG. 1. Necrosis of voluntary muscle fibers. $\times 600$.

FIG. 2. Testis, showing impaired spermatogenesis. Note multinucleated giant cells. $\times 220$.

lar dystrophy can be produced in a variety of mammals by eliminating vitamin E from an otherwise adequate diet. Since our preliminary report of paralysis in biliary fistula dogs,² it has been shown that vitamin E deficiency will cause dystrophy in dogs.¹³ Greaves and Schmidt¹⁴ have shown that vitamin E, like many other fat-soluble substances, is poorly absorbed from the intestine unless bile is present, and it seems likely that faulty absorption of vitamin E was responsible for the muscular dystrophy seen in our chronic biliary fistula dogs. Some of the animals with severe dystrophic changes were males, and these showed testicular degeneration of the type described in vitamin E deficiency. That faulty absorption of substances associated with the fats existed in these cases is evident further from the fact that a vitamin K deficiency developed. Also, extensive osteoporosis was present, indicating faulty absorption of vitamin D.

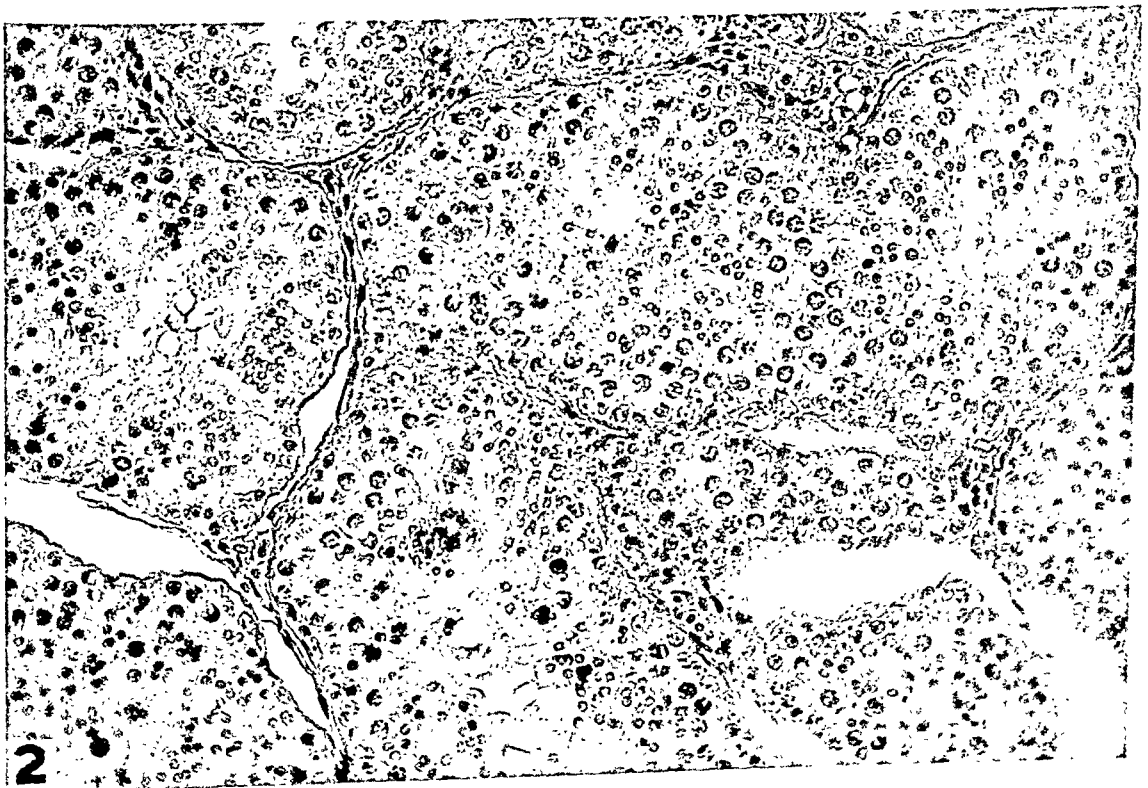
By analogy, it seems likely that this type of muscular dystrophy may be encountered at times in human cases in which there is difficulty in absorbing fat-soluble materials from the intestine. Patients having sprue, for example, are known to develop, at times, an extreme degree of muscular weakness. A muscular dystrophy on the basis of faulty absorption of vitamin E seems, on theoretical grounds, a possible cause for at least part of the muscular weakness.

SUMMARY

A nutritional muscular dystrophy, similar to that which has been produced in several mammals by eliminating vitamin E from the diet, is described in chronic biliary fistula dogs which were maintained on an adequate diet. It is suggested that the dystrophy is due to a vitamin E deficiency which results from faulty absorption in the absence of bile in the intestine.

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stilboestrol. The patients were then given 3 mg. of stilboestrol by mouth daily for 2 weeks. At the end of the 2 weeks, attempts were made to secure endometrial biopsies at the same time that the vaginal smears were taken. Only 4 patients yielded enough endometrium to be examined microscopically. The treatment was then continued for 5 weeks, until a total of 114 mg. of stilboestrol had been administered to each patient. Diagnostic endometrial biopsies were again attempted on all 10 patients by means of a Meigs' curette and material for study was secured from 8. Following this, the final phase of the study was continued for a period of approximately 4 months, during which time each patient had received a total of 324 mg. of stilboestrol. Vaginal smears were taken at weekly intervals and were evaluated according to the criteria established by Papanicolaou and Shorr.²

The experience of others³ has been that vaginal epithelial and endometrial responses occur after 2 mg. of stilboestrol have been administered daily for a period of 1 to 2 weeks. However, the reports referred to patients with a physiological menopause or with secondary amenorrhea. When our radium-treated cases were examined after 2 weeks, we found that we obtained no endometrial response nor changes in the vaginal smears. At the end of 5 weeks of treatment, a proliferative endometrium was observed and definite vaginal smear responses took place. It will be observed in referring to Table I that 6 cases showed definite proliferative

TABLE I
Biopsies and Vaginal Smears

	Endometrial biopsies					Vaginal smears
	Atrophic endometrium	Proliferative endometrium	Secretory endometrium	Cystic glands	None obtained	
Before radium	0	7	2	1	0	Not taken
After radium	3				7	Menopausal smear
Treatment 2 weeks (48 mg.)	4				6	No response
Treatment 5 weeks (114 mg.)	2	6			2	5 responded

responses of the endometrium after 5 weeks of treatment; 2 showed very slight response, designated as atrophic endometriums, and in 2 cases no biopsy was obtained due to a complete closure of the external os, preventing introduction of a curette.

ENDOMETRIAL RESPONSE TO DIETHYLSTILBOESTROL IN RADIUM-INDUCED MENOPAUSE*

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It is generally recognized that the menopause syndrome following radium-induced castration results in a clinical condition more difficult to treat than if it results from surgical castration or follows physiological involution. No report was found in which special attention had been paid to the effect of estrogenic substances on the genital tract in patients who had previously been treated with sufficient radium to produce a state of amenorrhea. Hence, it was decided to observe the anatomical changes in the uterus resulting from the use of radium and to study, subsequently, the influence of diethylstilboestrol¹ (4:4-dihydroxy-a: b-diethylstilbene)[†] at various intervals.

The age of the patients under observation varied from 42 to 50 years at the time of radium insertion. The group consisted of 10 patients who were suffering from bleeding dysfunction incidental to the oncoming menopause. Each had been exposed to 1200 to 1800 mg. hours of radium and one had received 2400 mg. hours of radium irradiation. Diagnostic curettements were done in all cases before radium was inserted in order to eliminate the possibility of the existence of malignancy. This also afforded an opportunity to study the histological appearance of the untreated endometrium. One to 2 years after the radium had been inserted and the bleeding had ceased, attempts were made to secure biopsies. One patient was curetted 5 months after the radium treatment. Vaginal smears were taken in all cases when the endometrial biopsies were secured in order to compare them with smears to be taken at subsequent intervals after treatment with

* Read before the Pittsburgh session of the American Association of Pathologists and Bacteriologists March 22, 1940.

† The stilboestrol used in this study was kindly supplied by Dr. Joseph A. Morrell of E. R. Squibb & Sons.

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tional phase. Only 2 cases showed tortuous glands in which secretory vacuoles were present; yet even these showed an intermingling with the changes of a proliferative phase—evidence of a failing corpus luteum. One patient showed a moderate degree of cystic distention approximating the Swiss cheese type of endometrium, while 7 cases showed a proliferative endometrium with no evidence of progestational effect on the glands.

After radium application, attempts were made to secure biopsies and in only 3 instances did successful curettements result. These showed an atrophic endometrium in which a few isolated glands were supported by a hyalinized stroma. In 7 cases no material was obtainable. After 5 weeks of treatment, 6 successful biopsies were obtained. These tended to duplicate the picture which was observed with the original curettements before radium was inserted. The glands were proliferative, the stroma showed congestion and edema, and the stroma cells were proliferative and hypertrophied. In a few cases some myometrium was secured and it was observed that the myometrial cells were hypertrophied and stimulated to proliferation.

It was extremely interesting to observe the type of reaction that was secured by stimulation with stilboestrol in consequence of the previous treatment with radium. If only vestiges of endometrial acini remained, the stilboestrol was sufficient to stimulate them to proliferation. The wall of the uterus in some of the cases showed dense hyalinization which did not respond to the stilboestrol but contained contiguous stimulated myometrium or endometrial glands or stroma. There were areas in which isolated muscle fibers had not been destroyed and were brought to life, so to speak, in the interstices of the hyalinized masses. In 1 case it was observed that the stroma cells of the endometrium had begun to be stimulated in 2 weeks, while in 5 weeks they showed approximation of the premenopausal state. This case demonstrated the earliest stimulation observed in the group studied.

DISCUSSION

Our studies, made on a series of cases in which an artificial menopause had been produced by radium, confirmed the belief that such cases are more refractory to stilboestrol than cases of surgical sterilization or those associated with normal physiological

Of the 8 patients who had patulous cervical canals, 3 (Table II) experienced cyclic bleeding during the course of the study. In all 3 cases the first period of vaginal bleeding occurred 6 weeks after treatment had been initiated. Subsequently, bleeding occurred in 2 cases at monthly intervals. This bleeding cannot be interpreted entirely as a type of withdrawal bleeding, since the treatment was

TABLE II
Bleeding Response to Stilboestrol

	No.	Dose	Treated	Vaginal bleeding
Patients with menopausal syndrome due to radium	10	324 mg.	4 months	3 cases
Patients with physiological menopausal syndrome	7	90-100 mg.	4-6 weeks	6 cases
Patients with surgical menopausal syndrome	11	Bleeding occurred in those patients who had had amputation of the fundus		

continuous, but apparently represented the result of a considerable degree of endometrial proliferation. In contrast to this observation it is interesting to observe that 6 out of 7 patients with a physiological menopause developed vaginal bleeding in the course of treatment. Also all of a group with a surgically-induced menopause in whom only a fundal amputation had been done, showed vaginal bleeding. These latter groups were observed in another study but were compared to the radium-induced menopause cases in order to evaluate the differences in physiological response. When we compare (Table I) the response of the vaginal epithelium, as evidenced by smears, with the changes that occurred in the endometrium, we find that the time required for the vaginal epithelium to respond to stilboestrol parallels that for the endometrial response.

MICROSCOPIC CHANGES

The sections of the endometrium taken from patients in whom bleeding dysfunction occurred before radium was inserted, exhibited a definite morphological character which is seen in bleeding associated with a failing corpus luteum. The glands all presented a marked degree of epithelial proliferation in which the cells were several layers in thickness and which showed the presence of mitotic figures. They were supported by a moderately congested endometrial stroma. Thus they presented the dual picture of endometrial glands during the follicular phase and an edematous, congested stroma similar to that seen in the progesta-

tends to produce a proliferative endometrium and stroma which duplicates the appearance of the endometrium before radium was inserted.

3. There is less tendency to produce bleeding in the radium-treated menopausal cases than in others.

4. Radium injures the stroma and myometrium as well as the glands. Stimulation by stilboestrol affects all three components.

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DESCRIPTION OF PLATES

PLATE 18

FIG. 1. Proliferative congested endometrium of a bleeding dysfunction case before radium treatment. $\times 100$.

FIG. 2. Endometrial response after 5 weeks' administration of stilboestrol 2 years after radium-produced atrophy. Had four periods of cyclic bleeding. $\times 100$.

processes. In the radium-treated cases we were dealing with the dual effects on the endometrium of the physiological regression incidental to menopause age, plus the destructive effects of radium. In the physiological menopausal cases the endometrium remained intact even though it became atrophic. The same consideration holds for whatever endometrium is left following partial hysterectomy, which may accompany ovariectomy. The response appeared to be related quantitatively to the intact endometrium that persisted.

The efficacy of radium or X-ray irradiation in bringing about a prompt amenorrhea in these cases can be explained when it is recalled that the irradiation acts on the nucleus of the cell and not on the cytoplasm. The normal cell is more vulnerable during the time of cell division than during the resting stage. Since patients with bleeding dysfunction exhibit a proliferative endometrium, in which there are numerous mitotic nuclei, the cells are extremely vulnerable to irradiation. In addition to bringing about a prompt cessation of bleeding, radium also produces a widespread destruction of cells. In the bleeding dysfunction cases that we studied, the majority (70 per cent) showed a proliferative endometrium in which there were numerous mitotic figures. Marked destruction of the glandular structures resulted as a consequence of the irradiation. The supportive tissue is more resistant to irradiation than glandular tissue and, consequently, this could be stimulated somewhat earlier than could the acini. This was made evident by the case which showed an early stromal response after 2 weeks of treatment. The only evident explanation of the occurrence of bleeding in only three cases under treatment, as compared to the much higher percentage in the physiological cases, is that endometrial stimulation by stilboestrol is inhibited in the radium-treated cases to a greater degree than in those not irradiated.

SUMMARY

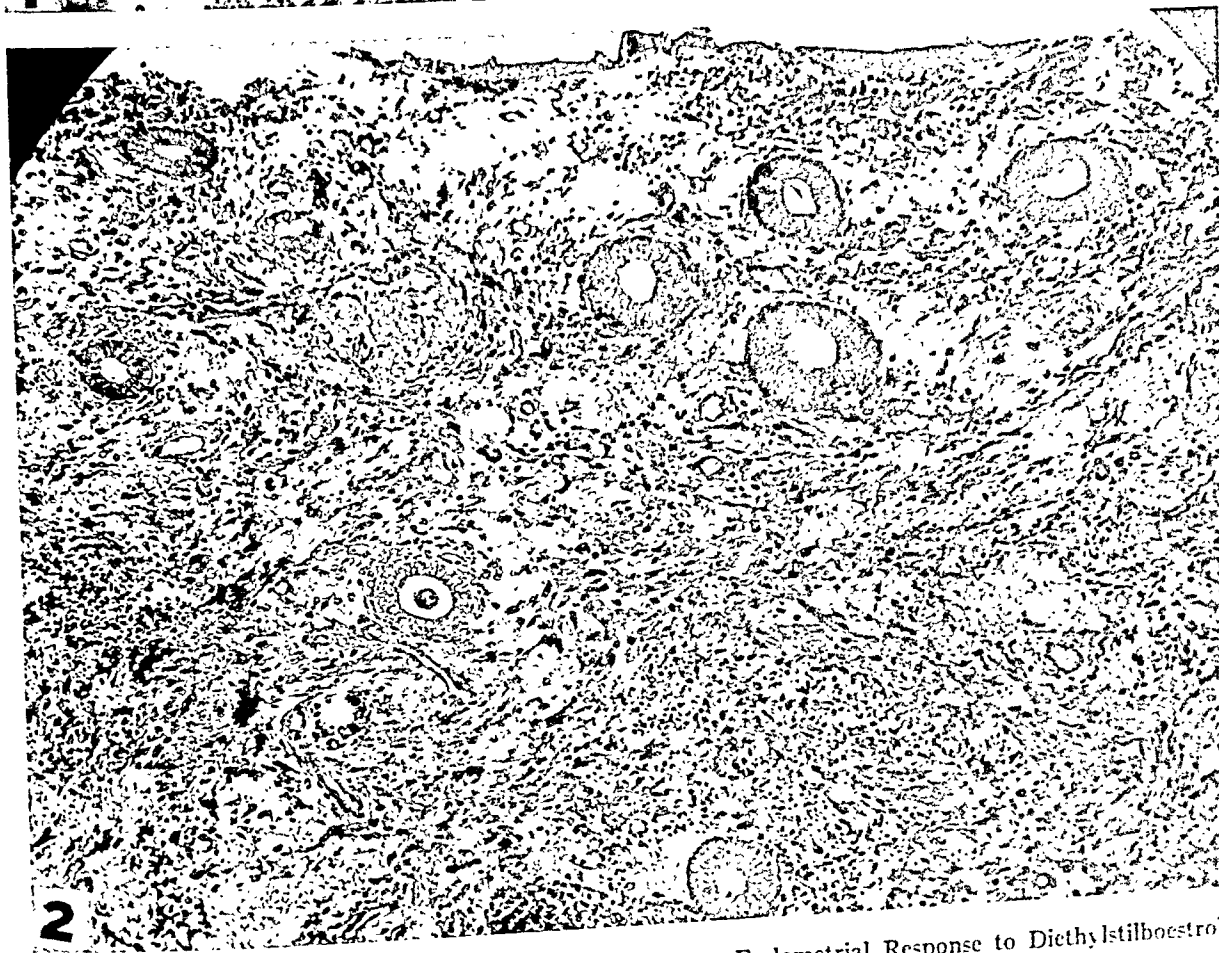
1. Diethylstilboestrol stimulates the glands, the stroma and the myometrium in radium-treated cases of bleeding dysfunction.

2. The endometrial and vaginal response to stilboestrol in radium-treated cases is delayed, and the dose required is greater, as compared to cases of physiological menopause. The stilboestrol

PLATE 19

FIG. 3. Proliferative endometrium associated with continuous menorrhagia. No progestational effect. $\times 100$.

FIG. 4. After 5 weeks' treatment with stilboestrol. Stimulated cells seen in interstices of hyalinized myometrium in upper left. Glandular epithelial stimulation in upper right. No cyclic bleeding occurred. $\times 46$.



Endometrial Response to Diethylstilboestrol

PLATE 20

FIG. 5. Stimulation of stroma cells after 2 weeks' administration of stilboestrol. Earliest evidence of stilboestrol effect. $\times 100$.

FIG. 6. Same case as Figure 1 after 5 weeks' administration of stilboestrol. Note glandular as well as stroma cell proliferation. $\times 200$.



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Endometrial Response to Diethylstilboestrol



another reason, therefore, for tumor mutation, and the experiments reported here represent one of our attempts to obtain information in this direction.

We are reporting two phases of our study: (1) an attempt to determine the susceptibility of our strain of rats to 1:2:5:6 dibenzanthracene; and (2) an attempt to determine whether such an agent is capable of inducing changes in implanted adenofibromas similar to those occurring spontaneously in the course of continued transplantations.

To our knowledge, the strain of rats used by us never has produced spontaneous sarcoma or carcinoma. We have stated elsewhere² that we believe that in this strain both mammary and genital tissues exposed to superphysiologic estrogenic stimulation are inherently protected against malignant degeneration.

EXPERIMENT I

The Susceptibility of Our Rats to Dibenzanthracene

To 37 young unimplanted male and female rats were given three or four injections of 1:2:5:6 dibenzanthracene at weekly intervals.* Twelve rats survived 100 days or more. Ten of these developed tumors at the site of injection (Table I). While the series lacked sufficient size and uniformity to allow any statistical deductions, it is apparent that dibenzanthracene is able to induce a high percentage of local sarcomas in our rats.

The sarcomas thus produced presented a wide range in time of appearance, 102 days being the shortest and 236 days the longest

TABLE I
*Occurrence of Induced Sarcomas in
Unimplanted Animals Treated with Dibenzanthracene*

A. Unimplanted males						
No. of Animals	Age at injection days	No. living 100 days	No. of induced tumors	Days to Appearance	Appearance to removal days	Tumor weight grams
11	34	2	1	199	34	56.0
20	102 to 156	9	8	102 to 220	2 to 52	0.7 to 56.3
B. Unimplanted females						
	days				days	grams
6	96 to 105	1	1	236	25	30.0

* Injections of 0.5 to 1.0 cc. of 0.4 per cent dibenzanthracene (E. K. Co.) in lard were given subcutaneously into the loose connective tissue beneath the shoulder, remaining well away from the site of the implanted tumor. Previous trials with intraperitoneal injections proved this method to be undesirable because of the markedly increased hazard due to toxicity.

EFFECT OF DIBENZANTHRACENE ON TRANSPLANTABLE MAMMARY ADENOFIBROMA OF THE WHITE RAT *

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Since 1928 we have been engaged in the study of growth behavior of mammary adenofibromas of white rats in relation to various phenomena of functional degeneration. Our main approach to the problem has been through serial transplantation of tumor tissue into a highly inbred strain of white rats originally derived from the Wistar stock. We reported elsewhere¹ that in certain instances it is possible to maintain the morphology and growth behavior of the original tumors through many transplantations but that, for reasons yet unknown, certain lines of transplanted adenofibromas change fairly abruptly into fibromas and sarcomas. This latter change offers an interesting problem in spontaneous malignant degeneration. The transition is invariably initiated by the gradual loss of glandular elements. In due time a pure connective tissue tumor forms, and ultimately differentiates into either a very mature fibroma or a viciously growing sarcoma. In isolated instances adenofibromas have assumed the morphologic appearance of adenocarcinoma but further transplantations have failed to prove functional malignancy. So far, all attempts to induce malignancy in adenofibromas by other means, such as rapid and frequent breeding or overdosing with growth hormone or huge doses of estrogenic substances, have been unsuccessful.² It therefore seems unlikely that the sarcomatous degeneration taking place during transplantation of our tumors can be explained on an endocrine basis. Neither do we think that the mere mechanical transference of tumor tissue from one host to another is the explanation. If it were, all adenofibromatous tissue transplanted by us would in time have changed into sarcoma, which has not been true. Some of our adenofibromatous tumor material has survived many transplantations since 1928 and still is morphologically and functionally similar to the original tumor. There must be

* Supported by a grant from Rockefeller Fluid Research Fund of Stanford University School of Medicine.

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TABLE III
Male Series
Treated Group

Animal No.	Body weight gain per day	Implanted tumor		Induced sarcoma		
		Final weight	Gain per day	Injection to appearance	Appearance to removal	Weight on removal
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>days</i>	<i>days</i>	<i>grams</i>
1600	0.19	32.36	0.140	180	22	46.00
1610	.05	—	—	152	36	108.75
1618	.60	2.40	.010	194	8	18.08
1591	.63	12.70	.059	152	36	39.00
1602	.46	4.24	.018	—	—	—
1604	.69	7.00	.030	229	35	106.32
1606	.55	5.81	.025	243	23	37.10
1608	.53	4.33	.019	215	4	2.84
Average	0.46	—	0.044	195	—	—

Control Group

Animal No.	Body weight gain per day	Implanted tumor	
		Final weight	Gain per day
	<i>grams</i>	<i>grams</i>	<i>grams</i>
1599	0.63	9.40	0.041
1601	.73	9.25	.040
1603	.51	2.03	.009
1605	.71	7.10	.031
1607	.58	6.16	.027
1609	.58	41.50	.180
Average	0.62	—	0.055

EXPERIMENT II

The Effect of a Cancerigenic Agent on the Behavior of a Benign Transplantable Adenofibroma

A richly glandular adenofibroma (Fig. 5) was implanted into 34 rats (19 females and 15 males) approximately 90 days old. One month later, after the implants were well established, 8 males and 8 females were given injections of 0.4 per cent dibenzanthracene exactly as described in Experiment I. The remaining implanted animals were set aside for controls. (One male died 52 days after implantation and was excluded.) Results are given in Tables II and III. The figures indicate that the growth of the implanted tumors in the treated males did not differ significantly from that in the untreated males. However, there was a definite retardation of growth in the treated females as compared with the controls (Table IV).

period. Once established, these tumors grew very rapidly and infiltrated muscle and surrounding tissue. Their rate of growth was similar to that of our sarcoma E I-2,¹ but differed from it by a greater tendency to invade. Microscopically, they differed in degree of anaplasia and mitotic activities, both of which are considerably greater in the chemically induced tumors (Figs. 1 and 2; 3 and 4). The dibenzanthracene tumors were as readily transplantable into other strain-related rats as our sarcomas. Serial transplantation did not alter their malignant behavior. They failed to produce distant metastases and in all instances occurred only at the site of injection. In this respect they resembled our sarcomas and, like them, killed by some unknown mechanism not dependent on size of metastatic growth.

TABLE II
*Adenofibroma Growth, Body Weight Changes and Occurrence of Induced Sarcoma
in Control and 1:2:5:6 Dibenzanthracene Groups*
Female Series
Treated Group

Animal No.	Body weight gain per day	Implanted tumor		Induced sarcoma		
		Final weight	Gain per day	Injection to appearance	Appearance to removal	Weight on removal
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>days</i>	<i>days</i>	<i>grams</i>
1572	0.32	24.33	0.180	173	29	60.00
1578	.11	79.00	.416	180	60	43.98
1592	— .18	50.09	.371	—	—	—
1615	.20	38.08	.280	125	36	64.20
1563	.25	22.48	.223	—	—	—
1565	.17	51.00	.375	271	36	90.00
1576	.15	41.00	.304	271	36	67.68
1613	.24	35.80	.188	292	15	7.80
Average	0.16		0.292	219		

Control Group

Animal No.	Body weight gain per day	Implanted tumor	
		Final weight	gain per day
	<i>grams</i>	<i>grams</i>	<i>grams</i>
1551	0.19	60.10	0.445
1564	.37	27.46	.272
1568	.16	—	—
1571	.24	93.43	.692
1573	.21	41.11	.305
1577	.07	93.80	.695
1594	.15	62.00	.614
1612	.33	26.60	.196
1614	.40	48.24	.478
1567	.07	93.50	.926
1569	.23	—	—
Average	0.22		0.514

taneous tumor formation. The frequency of spontaneous mammary adenofibromas has been but 0.3 per cent. Spontaneous malignant tumors have never been seen in this strain of rats.

The high incidence of chemically induced tumors indicates that our strain of rats is not completely refractory to malignant degeneration. But whatever this peculiarity may be, it seems to be confined strictly to local tissue reactions. In some ways this parallels the behavior of the tissues of our tumors. However, we do not believe that a mechanism as simple as a specific chemical stimulation is responsible for the sarcomatous changes occurring in our tumors. That the susceptibility to coal tar tumors does not necessarily bear a relationship to susceptibility to spontaneous tumors or tumor transplantability was shown by Andervont⁷ for mice.

The results of our second experiment further emphasize the remoteness of the possibility of a chemical agent causing malignant changes in transplantable mammary adenofibromas. Beyond depressing the rate of tumor growth in females, dibenzanthracene failed to induce morphologic changes in these tumors when injected at a distance. The toxicity of the chemical agent may have been concerned with the decrease in growth by disturbing body metabolism, as pointed out by Lees,⁸ but since this phenomenon was peculiar to females this cannot be the sole explanation. Our observations differ from those of others in the type of tumor used. It is evident that the influence of dibenzanthracene on a slowly growing adenofibroma may differ from that on the rapidly growing malignant tumors used by others because of the longer duration of exposure.

The effect of dibenzanthracene on tumor-bearing animals has been studied by several investigators. Haddow,⁹ in studying this relation to the Jensen rat sarcoma, found it to inhibit tumor growth. In other investigations¹⁰⁻¹⁴ these findings have been confirmed for various transplantable tumors in the rat. In mice, there are differences in susceptibility to cancerigenic hydrocarbons. Pybus and Miller¹⁵ and Haddow¹⁶ reported an inhibitory effect similar to that observed in rats, while Andervont¹⁷ noted an increased incidence of lung carcinoma in a strain of mice subject to spontaneous lung cancer. Taschner, Gottlieb, Spritzer and Plonskier¹⁸ reported a higher percentage of takes of Jensen rat

TABLE IV
Mean Daily Changes in Tumor Weight (Gain per Day)

	Controls	Treated	Difference	P*
Males	.055	.043	.012	.7
Females	.514	.292	.222	.027

* P = probability of difference as great or greater than that obtained by chance alone. See Fisher, R. A. *Statistical Methods for Research Workers*. Oliver and Boyd, London, 1934, Ed. 5, Chap. 5.

From long experience with these transplantable adenofibromas we know that the males commonly produce a tumor rich in fibrous constituents and poor in glandular components, while the females usually produce the opposite type. These characteristics evidently were not disturbed in the treated groups (Figs. 6-9). In both female groups the usual whorled pattern of an adenofibroma rich in glandular elements was maintained, and in none was there any evidence of unusual activity or of glandular or connective tissue disorganization.

The sarcomas developing at the site of dibenzanthracene injection were of the same character and occurred with the same frequency as those described in Experiment I. The interval between initial injection and first appearance of the tumor varied from 125 to 292 days. Tumors occurred in 7 of the 8 treated males and 6 of the 8 females. Microscopically, the sarcomas were identical with those of Experiment I.

DISCUSSION

Regardless of the presence of a benign implanted tumor, 1:2:5:6 dibenzanthracene induced local malignancies to the extent of over 80 per cent in our strain of rats. Other workers have obtained a wide range of difference in the susceptibility of different strains of rats to dibenzanthracene. Burrows, Hieger and Kennaway³ in 1932 found 15 tumors in 67 rats (22 per cent); Barry and Cook⁴ found 8 tumors in 20 rats (40 per cent); Shear⁵ in 1936 reported 11 tumors in a group of 18 rats (61 per cent); and Haagensen and Krehbiel⁶ found 26 tumors in 128 rats (20 per cent). A similar variation in the susceptibility of mice has been reported.

The susceptibility of rats of our strain to this carcinogenic agent is in marked contrast to their inherent resistance to spon-

5. Dibenzanthracene exerts an inhibitory effect on the growth of adenofibromas in the female rats.
6. It is not likely that spontaneous sarcomatous degeneration of transplanted mammary rat adenofibromas is the result of a chemical cancerigenic agent of the dibenzanthracene type.

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sarcoma in mice treated with methylcholanthrene. Appel, Strauss, Kolischer and Necheles¹⁹ found that dibenzanthracene produced a more rapid growth of the Brown-Pearce carcinoma of rabbits, with an increase in metastatic invasion. Rabbits and mice, therefore, may differ from rats in degrees of susceptibility to chemically induced tumors. Our findings in a cancer-free strain of rats prove that the inherent protection against malignancy can be overcome to some extent but that such response is confined to tissues in contact with the agent, since implanted adenofibromas fail to undergo malignant change, although capable of doing so spontaneously.

In comparing the sarcomas induced by dibenzanthracene with those occurring in the course of transplantation of our adenofibromas it is evident that the former possess a greater degree of malignancy. However, they resemble each other in that they fail to produce distant metastases, recur after removal and yield 100 per cent takes upon subsequent transplantation. The malignant degenerative processes of the two, on the whole, are similar, but in view of the lack of influence of dibenzanthracene on the implanted benign tumors it is not likely that a cancerigenic agent of the dibenzanthracene type is the factor causing sarcomatous degeneration of the benign mammary adenofibromas transplanted by us into a strain of rats refractory to spontaneous cancer.

CONCLUSIONS

1. The subcutaneous injection of 1:2:5:6 dibenzanthracene in a strain of rats refractory to spontaneous cancer produces sarcomas in over 80 per cent of animals surviving the injection period 100 days or more.

2. The sarcoma induced by dibenzanthracene is readily transplantable through six generations, fails to metastasize, and microscopically is more anaplastic than sarcomas derived from the adenofibromas.

3. The presence of a previously implanted benign tumor has no effect on the incidence or character of these induced sarcomas.

4. Dibenzanthracene fails to induce malignant changes in a transplantable mammary adenofibroma known to undergo spontaneous malignant degeneration.

DESCRIPTION OF PLATES

PLATE 21

FIG. 1. Dibenzanthracene induced sarcoma (low power). $\times 100$.

FIG. 2. Dibenzanthracene induced sarcoma (high power). $\times 450$.

FIG. 3. Sarcoma E I-2 (low power). $\times 100$.

FIG. 4. Sarcoma E I-2 (high power). $\times 450$.

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fully the value of the chorio-allantoic membrane of fertile eggs as a medium for experimental pathologic problems, emphasizing in particular "the conception of organic differentiation" and "the selective influence of living cells in certain infective processes" as characterized by Goodpasture.¹

The method of fertile egg inoculation as used today, however, may be chiefly attributed to the unceasing efforts of Goodpasture and his associates. Adapting the technic described by Clark⁵ in 1920, Woodruff and Goodpasture⁶ in 1931 obtained characteristic histopathologic lesions with fowlpox virus on the chorio-allantoic membranes. Following this initial paper, Goodpasture, in collaboration with various members of his department, further developed the practicability of the technic to a point of reliable experimental procedure, especially as improved by Buddingh.⁷ The experiments, thus started, were extended to include viruses (as shown also by Burnet⁸ in a number of instances), bacteria, *Rickettsiae*, and a spirochete,⁹ and in June 1939¹⁰ there appeared a preliminary report on the successful inoculation and "take" with fungi.

TECHNIC

As employed here, the method is essentially the same as that used by Goodpasture and Buddingh⁷ and by Burnet⁸ for cultivating viruses in the chorio-allantoic membrane of the chick embryo. The coverslip method was employed in preference to the shell-flap method. The former is best adapted for the study of developing lesions.

Because of the slowness of development of fungi, it was found advisable to use eggs incubated 10 to 14 days, as advocated by Goodpasture,¹ depending on the type of organism to be used. Yeastlike organisms show a development time of approximately 5 to 7 days, whereas filamentous forms have a longer evolution time, varying from 5 to 11 days. For such, therefore, it was necessary to use eggs incubated only 10 days.

After inoculation, the eggs were placed in a regular bacteriologic incubator and maintained at a constant temperature without turning. For fungi it was found best to maintain the temperature at approximately 33° C. The eggs were examined daily and, when growth had developed on the membrane for a sufficient period, the

THE CHORIO-ALLANTOIC MEMBRANE OF THE DEVELOPING CHICK AS A MEDIUM FOR THE CULTIVATION AND HISTOPATHOLOGIC STUDY OF PATHOGENIC FUNGI *

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In the search for ways to hasten diagnostic procedures and to improve therapeutic measures, microbiologists are employing unprecedented means of biologic investigation. One such method is the use of the chorio-allantoic membrane of the developing chick for the growth and determination of various microorganisms, including numerous viruses, Rickettsiae, bacteria and spirochetes. The use of this structure in the fertile egg for the isolation, identification and further study of organisms has not, in any great measure, been used for the study of fungi, except as mentioned by Goodpasture¹ in explaining the application of the membrane in investigating infectious processes and in examining the fungus-contaminated chorio-allantois.

Egg contents have been employed as enriching substances for cultivation for many years, chiefly in bacteriology and only to a very limited extent in mycology. One of the earliest uses of the egg as a medium for the cultivation of fungi was that of Wolff and Israel² who, in 1891, obtained pure cultures of *Actinomyces* by the inoculation of pus from a retromaxillary nodule into raw and partially boiled hen's and pigeon's eggs.

The use of fertile eggs, however, seems to have had its start with the work of Levaditi³ in 1906, who infected chick embryos with a spirillum of fowls in continuation of some experiments started by Borrel in 1905. In addition to making other important observations, Levaditi pointed out the significant relationship between living embryonic tissue and susceptibility to infection as found in spirillosis. Later, in 1911, Rous and Murphy,⁴ in studying a neoplasm of chickens (Rous sarcoma) which had been discovered by Rous in 1911, were able to demonstrate very success-

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G. *Actinomyces bicolor* (actinomycosis)

H. *Monosporium apiospermum* (maduromycosis, Madura foot)

I. *Phialophora verrucosa* (chromomycosis)

When possible, young subcultures of several days' growth were used in order to obtain active proliferation. Several of the organisms had been isolated from human lesions only a short time prior to experimentation.

LESIONS OF THE CHORIO-ALLANTOIC MEMBRANE

I. Superficial dermatomycoses (superficial desquamation, pityriasis)

A. *Malassezia furfur* (Robin) Baillon, 1889.

Eggs were incubated 11 days and observed 5 days after inoculation. Macroscopically, the lesion on the chorio-allantoic membrane, including the inoculum, had the appearance of a nodule, somewhat irregular in outline. There was a slight opacity in the membrane around the periphery of the nodule. The embryos were alive.

Microscopically, a section of the membrane through the nodule showed it to be made up of the inoculum of fungus. Underlying the fungous growth and fading into the ectoderm was a translucent, more or less granular, substance which stained with eosin. At the junction of this layer and the ectoderm there was a heavy infiltration of monocytes, indicative usually of a mild infection. The monocytes extended to the edge of the nodule where they were seen in larger clusters. On the outer surface of the nodule were seen clusters of red blood cells. The ectoderm appeared thickened in some regions underlying the inoculum, due to a marked proliferation of ectodermal cells. Just underlying the area of marked ectodermal proliferation were seen fibroblasts and groups of proliferating ectodermal cells, interspersed among which were red blood cells and leukocytes. Beneath this area in the mesoderm were small groups of leukocytes and mesenchymal cells. In areas of the mesoderm, but not directly associated with the inoculum, were large groups of ectodermal cells showing pearl formation, indicating hyperkeratinization, and infiltrated with

shell was cut just below the surface of the chorio-allantois and removed. A small amount of Zenker's fixative (with 5 per cent glacial acetic acid) was dropped on the inoculated area to harden the membrane, which was then removed with a pair of fine scissors with curved points and placed in Zenker's solution. The membranes were then embedded in paraffin, sectioned and stained with Loeffler's methylene blue and eosin, cleared in xylol and mounted on slides.

MATERIALS*

The organisms cultured and the diseases which they produce are listed as follows in the order of presentation:

- I. Superficial dermatomycoses (superficial desquamation, pityriasis)
 - A. *Malassezia furfur* (pityriasis or tinea versicolor)
 - B. *Pityrosporum ovale* (seborrheic dermatitis)
- II. Dermatomycoses (epidermic and dermic lesions)
 - A. *Trichophyton gypseum* (trichophytosis, tinea, ring-worm)
 - B. *Epidermophyton inguinale* (epidermophytosis, athlete's foot, tinea cruris)
 - C. *Achorion Schoenleini* (favus)
 - D. *Microsporum canis* (microsporiasis)
- III. Cutaneous, subcutaneous, mucous membrane and internal organ involvement (localized cutaneous lesions, granulomata, deep-seated ulcerative lesions, mucous membrane plaques, lymph stream invasion with dermic and subsequent epidermic involvement, and visceral or generalized diseases)
 - A. *Monilia albicans* (moniliasis)
 - B. *Geotrichum versiforme* (geotrichosis, mucous membrane and bronchial lesions)
 - C. *Zymonema dermatitidis* (blastomycosis)
 - D. *Cryptococcus hominis* (cryptococcosis, torulosis)
 - E. *Coccidioides immitis* (coccidioidal granuloma)
 - F. *Sporotrichum Schencki* (sporotrichosis)

* The binomial nomenclature of the fungi used in this work follows that of Dodge, Carroll William. Medical Mycology, Fungous Diseases of Men and Other Mammals. C. V. Mosby Company, St. Louis, 1935.

ticularly at the periphery of the inoculum adjoining the membrane. In addition, a large number of red blood cells were seen at the periphery and somewhat on the surface of the growth. The ectoderm was markedly thickened and hyperplastic in areas, but was largely replaced by inflammatory granulation tissue arising from the mesoderm. A layer of fibroblasts lay just beneath the replaced area of ectoderm. The mesoderm was markedly edematous, showing numerous islets of ectodermal cells, a huge number of fibroblasts, leukocytes and monocytes with a perivascular infiltration, endothelial cell hyperplasia and increased number of distended capillaries. The marked edema in the area underlying the inoculum had resulted in a greatly thickened membrane. The part of the entoderm lying directly under the center of the inoculum was also very hyperplastic with elongated papillae merging into the normal portion of the layer. The whole reaction varied in intensity in relation to the center of the inoculum, being very marked in the center of the membrane and becoming less toward the edge of the fungous growth.

The fungous elements adjoining the ectoderm had the morphology and characteristics of those seen in culture. At the periphery of the growth, however, the fungus appeared as fine filaments and small, spherical spores without the large round cells seen in culture (Fig. 4).

B. *Epidermophyton inguinale* Sabouraud, 1910.

Eggs were incubated 13 days and observed 8 days after inoculation. Macroscopically, the membrane was thickened by a marked confluent growth of the inoculum with aerial mycelium. Surrounding the inoculum there was a grayish, thickened opaque area. The embryos were dead.

Microscopically, the lesion simulated closely a "traumatic ulcer" showing loss of continuity of the ectoderm and replacement with inflammatory tissue arising from the mesoderm. The ectoderm at the margins of the inoculum was greatly thickened, becoming normal beyond the zone of mesodermal edema which underlay the fungous growth. Necrosis was apparent in the area of the replaced ectoderm. Here there were leukocytes in stages of degeneration, fibroblasts, and a marked increase in capillaries packed with red blood cells. Immediately above this area numer-

leukocytes and some red blood cells. The entoderm appeared unaltered.

The inoculum itself exhibited a pink-staining, translucent, granular exudate with the fungi staining only with eosin in the center and base of the growth. The exudate merged with the ectoderm. The fungi at the periphery, however, showed a well developed growth which stained with methylene blue and had the characteristic appearance of the spherical cells and filaments seen in scrapings of a human lesion (Fig. 1).

B. *Pityrosporum ovale* (Bizzozero) Castellani and Chalmers, 1913.

Eggs were incubated 13 days and observed 5 days after inoculation. Macroscopically, the membrane showed some discrete, fine opacities over its surface. The embryos were alive.

Microscopically, groups of ovoid to spherical, budding, yeast-like organisms were seen on the ectoderm (Fig. 2). The ectodermal layer showed some proliferation and thickening, with vacuolated cells with a few inclusions of the sort that have been termed pseudo-inclusions by some authors. In places the organisms had invaded the ectoderm. The mesoderm was hyperplastic, and infiltrated by ectodermal cells, many monocytes, red blood cells and a few leukocytes. There was some endothelial hyperplasia. The entoderm was very slightly changed, if at all.

II. Dermatomycoses (epidermic and dermic lesions)

A. *Trichophyton gypseum* Bodin, 1902.

Eggs were incubated 13 days and observed 5 and 8 days after inoculation. Macroscopically, the growth on the membrane appeared as confluent colonies of aerial mycelium (Fig. 3). The membrane showed a grayish infiltrate, appeared greatly thickened immediately surrounding the macroscopic growth, and was white in color. The embryos were alive.

Microscopically, the growth on the chorio-allantois and the reaction of the tissue simulated very closely a "traumatic ulcer." The ectoderm had lost its identity in the central area underlying the inoculum, having become infiltrated with a layer of monocytes which lay between the fungous growth and the membrane. Many monocytes were interspersed among the fungous elements, par-

cells present and an edematous mesodermal infiltration associated with them (Fig. 8). The base of the inoculum, closely applied to the membrane, was infiltrated with monocytes, leukocytes and red blood cells. The mesoderm showed numerous fibroblasts and leukocytes, some monocytes, many capillaries with red blood cells, proliferating ectodermal cells and eosinophil-like cells. These various cells were more numerous in the region underlying the fungous growth and became fewer in the adjacent normal area. The entoderm beneath this infiltrate was hyperplastic.

The fungous growth was infiltrated by leukocytes and red blood cells. The fungus showed the characteristics of the organism in culture at the base of the growth, but took on the parasitic characteristic of short filaments at the periphery of the growth and in isolated regions where a small amount of the fungus was associated with the membrane (Fig. 7).

D. *Microsporium canis* Bodin, 1904.

Eggs were incubated 10 days and observed 5 days after inoculation. Macroscopically, the membrane showed a thick growth at the inoculum which was raised at the periphery with a grayish infiltrate in the membrane. The embryos were dead and the yolk was murky. The membranes were thickened.

Microscopically, the membrane exhibited a marked reaction to the presence of *M. canis*, the whole appearing very much as a "traumatic ulcer" as shown with several other fungi. The fungous growth extended along the surface of the broken ectoderm, which was replaced by cells of the inflammatory granulation tissue which had migrated from the mesoderm. The ectoderm, in the intact areas and particularly at the edges of the fungous growth, was much thickened and showed proliferating islets or groups of ectodermal cells extending into the edematous region at the base of the translucent, granular, fungus-invaded material adjoining the inflammatory area (Fig. 9). The base of the fungous growth was heavily infiltrated with monocytes, red blood cells, leukocytes and some degenerated cells. These cells were also interspersed among the clusters or islets of ectodermal cells of the discontinuous ectoderm.

The region of the mesoderm just below the necrotic ectodermal layer showed compact massing of fibroblasts and leukocytes,

ous monocytes formed a layer which was interspersed with the fungous elements. The mesoderm showed marked edema, cellular proliferation and migration of cells with many fibroblasts and ectodermal cells in islets or whorls in active proliferation. There were also groups of leukocytes, dilated capillaries, red blood cells, monocytes and occasionally eosinophil-like cells. The fibroblasts were more noticeable just under the inoculum. However, upon examination of the mesoderm on either side of the inoculum, leukocytes were observed in large numbers, decreasing as the normal area of the membrane was approached.

The entoderm, associated with the edematous area of the mesoderm, exhibited an increased proliferation which diminished in the direction of the nonaffected area of the mesoderm.

The organism, *E. inguinale*, as it appeared on the membrane, consisted of spherical cells which took only the eosin. Scattered among them were numerous monocytes, leukocytes and red blood cells. Going outward, toward the periphery of the inoculum, there were seen fewer spherical cells, but filaments were present which took the methylene blue stain in addition to the eosin. At the periphery of the inoculum, filaments and fuseaux were encountered chiefly, with some small round cells. But in sections of the ectoderm, where the organism was found in small collections, *i.e.*, in the area surrounding the inoculum, the fungus had invaded the ectoderm, reverted to its parasitic morphology as seen in human lesions and caused a thickening of the ectodermal layer (Figs. 5 and 6). The ectodermal cells had lost their normal morphology and had become degenerated and somewhat cornified, with a subsequent desquamation. In short, this reaction simulated a typical epidermophytosis.

C. *Achorion Schoenleini* (Lebert) Remak, 1845.

Eggs were incubated 12 days and observed 5 days after inoculation. Macroscopically, the membrane exhibited a growth consisting of aerial mycelium with a hazy grayish infiltration in the membrane, rather similar to that of *T. gypsum*. The live embryos showed large gas bubbles in the chorio-allantois, which was a characteristic found in all eggs inoculated with *A. Schoenleini*.

Microscopically, the ectoderm underlying the fungous growth was discontinuous, with marked proliferation of the ectodermal

character was maintained elsewhere. In places the ectodermal cells were flattened and elongated with evidence of monocytic invasion overlying this layer and invading the fungous growth. In other areas the ectoderm seemed to cornify in layers, on the surface, in the act of desquamation, while the underlying ectodermal cells were actively proliferating in scattered whorls. A fine, granular exudate was present on the desquamating ectoderm and in numerous areas the budding cells had invaded the greatly thickened, cornified ectoderm.

The reaction of the mesoderm to *M. albicans* was remarkable. In the areas of greatest activity, *i.e.*, under the marginal ectodermal thickening, there were whorls of actively proliferating ectodermal cells extending into the mesoderm, some appearing as elongated projections. Fibroblasts occurred just beneath the ectoderm, with numerous accumulations of leukocytes in whorls, often showing degeneration. There were many thrombosed capillaries. Most interesting, however, was the formation of many pearls of apparently ectodermal origin indicative of hyperkeratinization (Figs. 12 and 13). This process was analogous in all respects to that in infection of human epithelium with the same organism. The whole picture was one of edema with increased cellular activity and resultant thickening of the membrane in the involved area. The entoderm was not significantly altered except for slight thickening in the areas of mesodermal and ectodermal response, with some accumulation of leukocytes on its mesodermal surface.

This fungous growth is of interest also since the inoculum adjoining the ectoderm, except at the surface of the growth, induced the formation of an exudate, probably arising from the chorio-allantois, which was pink staining and within which could be seen filaments of the organism, red blood cells, monocytes and a few budding, yeastlike cells. At the periphery of the growth, however, the organisms were almost exclusively budding, and scattered among them were monocytes and red blood cells and the exudative, granular material (Fig. 10). The fungus here was of the type seen in human lesions.

B. *Geotrichum versiforme* Moore, 1934.

Eggs were incubated 12 days and observed 5 days after inoculation. The membrane macroscopically showed grayish yellow,

which seemed to be migrating toward the center of the overlying fungous growth. The mesoderm itself showed a marked cellular accumulation in this region and a generalized edema in the area of the fungous growth. The cellular infiltration consisted of large numbers of fibroblasts, leukocytes and red blood cells. There were many capillaries, some monocytes, and actively proliferating whorls of ectodermal cells surrounded in most cases by leukocytes. The fibroblasts were most numerous near the ectoderm, whereas large groups of leukocytes were prevalent deeper in the mesoderm and closer to the entoderm. The entoderm was somewhat thickened, but proliferation was not marked.

The fungous growth was seen in the translucent, granular material adjoining the ectoderm as filaments and spores with some monocytes scattered throughout. At the periphery of the growth the fungus consisted of short filaments and numerous, somewhat encapsulated spores. Eggs inoculated with a freshly isolated culture exhibited characteristic filaments and fuseaux typical of *M. canis* in culture.

III. Cutaneous, subcutaneous, mucous membrane and internal organ involvement (localized cutaneous lesions, granulomata, deep-seated ulcerative lesions, mucous membrane plaques, lymph stream invasion with dermic and subsequent epidermic involvement, and visceral or generalized diseases)

A. *Monilia albicans* Zopf, 1890.

This organism is likewise referred to as *Syringospora albicans* (Robin) Dodge, 1935.

Eggs were incubated 12 days and observed 6 days after inoculation. Macroscopically, the membrane showed diffuse to confluent grayish, opaque plaques, thicker than the apparently unaffected areas. The embryos were dead.

Microscopically, the membrane showed hyperactivity indicative of a marked response to the foreign organism (Fig. 11). The ectoderm was thickened, particularly at the margins of the lesion. Here there was active proliferation of the ectodermal cells with an infiltration of leukocytes, monocytes and red blood cells, some in various stages of degeneration. The ectoderm was not continuous in the area associated with the fungous growth, although its

various layers. There was a marked monocytic infiltration in the mesoderm with some whorls of ectodermal proliferation and some leukocytes. The fungus had the characteristics of the usual cultural growth, but showed, on the fifth day, the beginning of spherical cell formation (Fig. 16).

Membranes examined 10 days after inoculation were extremely thin and broken, showing ectodermal proliferations and cornification, with an almost complete loss of mesoderm. Scattered over the membrane were small nodules which on microscopic examination were found to be composed of several zones. There was some ectodermal thickening at the margins of the fungus growth. The region of the nodule adherent to the thin, cornified membrane showed a layer of monocytes. The central portion of the nodules was seen to consist of budding, thick-walled, yeastlike cells, comprising the parasitic type of *Zymonema dermatitidis* (Fig. 17). The organism in 10 days thus showed a reversion to a parasitic rôle.

D. Cryptococcus hominis (*Cryptococcus histolyticus*)
(Busse) Vuillemin, 1901.

Eggs were incubated 11 days and observed 5 days after inoculation. Macroscopically, there was a grayish, moist mat over the membrane with resultant thickening. The embryos were alive.

Microscopically, the chorio-allantois showed little response to the fungus except for a thickening of the ectoderm where it came in contact with the organism and a leukocytic infiltrate in the mesoderm which was not marked. The entoderm was not affected. The fungus-affected area exhibited a marked infiltration of monocytes with some red blood cells. The organism, *C. hominis* or *C. histolyticus*, was seen as a budding, yeastlike, mucoid-encapsulated cell, characteristic of the parasitic stage of the organism (Fig. 18).

E. Coccidioides immitis Stiles, 1896.

Eggs were incubated 13 days and observed 7 days after inoculation. Macroscopically, the membrane showed grayish yellow, thickened plaques which were somewhat confluent. The embryos were alive.

Microscopically, the chorio-allantois showed little response to

thickened plaques, some confluent, others diffuse, producing a thickened membrane. The embryos were dead.

Microscopically, there was a marked edematous reaction throughout the fungus-invaded areas and in the membrane (Fig. 15). The reaction simulated an ulcer to a certain extent, with the ectoderm broken in parts and replaced with inflammatory migrating cells from the mesoderm and from the proliferating ectoderm. The ectoderm was greatly thickened in areas with degenerated cells, red blood cells, monocytes and leukocytes. The marginal regions of the fungous growth on the ectoderm showed proliferation and thickening of the ectoderm. The mesoderm was edematous with many thrombosed capillaries, whorls or islets of proliferating ectodermal cells and several ectodermal pearls. Just beneath the ectoderm were fibroblasts and leukocytes with a number of small thrombosed capillaries. Scattered throughout the membrane were red blood cells and numerous basophilic leukocytes occurring in clusters or singly. The entoderm was thickened and proliferated below the regions of active ectodermal reaction, with many fibroblasts and capillaries in the adjacent mesoderm.

The fungous growth on the ectoderm was infiltrated by many inflammatory cells, such as leukocytes in various stages of degeneration, red blood cells, monocytes and some ectodermal cells. In several areas the fungus, *G. versiforme*, was composed of filaments and rectangular to ovoid cells. When associated with the membrane or the inflammatory cells of the membrane, the cells were the type seen in human tissue, somewhat rectangular to spherical or ovoid (Fig. 14).

C. Zymonema dematitidis (Gilchrist and Stokes) Dodge,
1935.

Eggs were incubated 12 days and observed 5 and 10 days after inoculation. Macroscopically, eggs inoculated for 5 days showed a mat of aerial mycelium on the membrane growing as a colony. The embryos were alive. Inoculated eggs observed after 10 days showed rather dry membranes, fairly thin and somewhat broken up. Distributed over the intact membrane were nodules which were yellow in color. The embryos were dead.

Microscopically, the younger membranes (5 days) showed an invasion by the fungus with a consequent loss in character of the

monocytes. The mesoderm showed leukocytes scattered throughout, many of them degenerated, and some monocytes and fibroblasts. The entoderm was somewhat thickened, necrotic in areas and somewhat broken through with a granular exudate on some areas of its inner surface. On its outer surface was present the same granular exudate, although more abundant, with numerous leukocytes, many degenerated.

The fungous inoculum was very interesting, having three zones (Fig. 21). At the base of the inoculum, adjoining the cornified ectoderm, were monocytes which were found in greater number at the margin of the growth. They were seen in groups dispersed through the fungous growth. The base of the inoculum was made up almost exclusively of round spores of the fungus. The center of the inoculum showed a translucent, granular substance which stained with eosin. Dispersed through this area were the fine, branching filaments of the organism, with some conidia. This region was flanked by spores and monocytes. The periphery of the inoculum showed clusters of the cigar-shaped cells of *S. Schencki*, the parasitic cells of the fungus (Fig. 22).

G. *Actinomyces bicolor* Trolldenier, 1903.

Eggs were incubated 11 days and examined 5 and 8 days after inoculation. Macroscopically, the membranes may show a single inoculation with a densely opaque area surrounding the implant and a lighter opaque area extending from the denser region (an ectodermal involvement) (Fig. 23), or they may show small, diffuse nodules on a densely opaque area with *Actinomyces bovis* (Fig. 24). The embryos were alive.

Microscopically, the chorio-allantois showed a marked reaction to the fungus, with edema and thickening of the membrane. The ectoderm associated with the growth was greatly thickened and proliferative, with a marked infiltration of monocytes, leukocytes and red blood cells, some of which had degenerated. The ectoderm was irregular and broken in areas where the infiltration was particularly heavy. The fungous growth on this layer was irregular in outline and was made up of fine, branching filaments with spore formation, many of the filaments having invaded the ectoderm itself (Fig. 25). The growth was also infiltrated with monocytes, leukocytes and some red blood cells. The mesoderm showed inflammation and edema with an invasion of many whorls

the organism except for a thickening of the ectoderm where it came in contact with the fungous growth. In some areas the ectoderm showed necrosis with an invasion of leukocytes which had undergone degeneration. The mesoderm in some areas showed focal invasion with leukocytes as well as scattered leukocytes throughout. The entoderm appeared unaltered. The surface of the membrane, however, showed a granular exudate in areas both on the ectoderm and entoderm, with numerous red blood cells. On the ectodermal surface, in addition, there was a more or less translucent, granular exudate which appeared in layers or striations, staining intensely with eosin (Figs. 19 and 20). This material covered most of the ectodermal surface and showed many red blood cells. Within this material, *C. immitis* could be seen in various stages of development, with the formation of the characteristic endosporulating cells of the parasitic stage of the fungus. The inoculated filaments were first converted into arthrospores, and then into spherical cells which enlarged and developed endospores. In 7 days on the chick membrane the organism had shown a reversion to its parasitic rôle which in animals usually takes a much longer time.

F. *Sporotrichum Schencki* Matruchot, 1910.

Eggs were incubated 13 days and observed 10 days after inoculation. Macroscopically, the membrane showed grayish, somewhat yellowish, thickened areas on its surface, somewhat raised in parts with a hazy infiltrate in the surrounding area. Embryos were dead.

Microscopically, the picture was somewhat confusing. The membrane showed edema with a large number of red blood cells, leukocytes, migrating cells, fibroblasts and monocytes, many showing degeneration. The ectoderm in many areas was necrotic and presented an inflammatory infiltration. In other areas, just underlying the heaviest growth of the inoculum, the ectoderm showed marked proliferation at its base and cornification. Just underlying the cornified ectoderm and displacing part of the mesoderm was a granular infiltration with groups of the cigar-shaped, parasitic cells of *S. Schencki* scattered throughout. Adjoining the exudate and extending into the mesoderm were degenerated leukocytes, some fibroblasts, red blood cells, and some

tion to the presence of the fungus, with edema, inflammation and resultant thickening and proliferation of the various layers. The ectoderm showed marked hyperplasia and proliferation with an invasion by the fungus, *P. verrucosa* (Fig. 29). There was a heavy infiltration of monocytes in the region where the fungus invades the ectoderm. The invaded ectoderm showed whorls of proliferating ectodermal cells which extended into the edematous mesoderm. The region underlying the involved ectoderm had marked ectodermal proliferation and migrating fibroblasts, and scattered among these were leukocytes and thrombosed capillaries. The mesoderm showed an inflammatory process with numerous leukocytes, whorls of ectodermal cells and capillaries which extended down to the entoderm. The entoderm was very little affected in spite of the reaction in the other two layers.

The fungous growth on the ectoderm consisted of an outer growth of loose filaments and round cells. The inner area had a somewhat translucent, granular material which stained with eosin and within which were seen the hyphae, round cells and some phialides of *P. verrucosa*.

SUMMARY AND CONCLUSIONS

A number of fungi representative of the causative agents of various types of lesions which develop in man were successfully inoculated in the chorio-allantoic membrane of the developing chick to determine the effect on the fungi and the reactivity of the chorio-allantois to the parasites. Fertilized eggs that were incubated 10 to 14 days were used in this study. Inoculations were made directly on the chorio-allantoic membrane. Macroscopically, the lesions manifested themselves as thick or thin, white, grayish or grayish yellow to light brown, confluent or discrete plaques on the membrane, depending on the type of organism used.

Microscopically, the membrane reacted in the form of nodules, ulcers, superficial growths and hyperplastic lesions showing varying degrees of proliferation. There was increased activity in most cases as evidenced by intense infiltration of ectodermal cells, red blood cells, fibroblasts, leukocytes and monocytes, with inflammatory changes in the mesoderm and ectoderm, and in some cases in the entoderm, and marked edema at the sites of fungous growth. This resulted in a thickening of the membrane which was in-

of proliferative ectodermal cells. These in turn were surrounded by numerous leukocytes. The leukocytes were seen in large numbers throughout this layer, as well as fibroblasts and thrombosed capillaries: Except for a few sections that were somewhat thickened, the entoderm did not seem to be affected.

H. *Monosporium apiospermum* Saccardo, 1911.

Eggs were incubated 13 days and observed 5 days after inoculation. Macroscopically, the membrane appeared considerably thickened in the affected areas, showing thick, grayish, opaque infiltrations either confluent or diffuse (Fig. 26). The embryos were alive.

Microscopically, there was very little differentiation of any of the layers of the membrane, since the fungus had grown through and involved the whole chorio-allantois (Fig. 28). The result was a thickened growth which was differentiated only according to the growth of the fungus. Dispersed through the affected membrane, however, in regions which the organism had not invaded completely, were seen groups of monocytes, some leukocytes and red blood cells. In other areas, within the membrane, were seen groups of single spores of *M. apiospermum* (Fig. 27).

The growth appeared in various layers. The peripheral growth stained with methylene blue and was seen as a loose network of fine filaments and small spores. In other areas there was a growth outward, onto the surface of the membrane, showing clusters of larger spores, more typical of *M. apiospermum* in culture. The second layer consisted of closely interwoven hyphae which stained only with eosin. The third area consisted of loosely intertwined and growing mycelium with clusters of spores comparable in size to those seen on the surface of the membrane. The growth of the fungus and the resultant change in the membrane indicated, in a measure, the type of pathology provoked in humans—that of a granuloma.

I. *Phialophora verrucosa* Thaxter, 1915.

Eggs were incubated 12 days and observed 5 and 8 days after inoculation. Macroscopically, the membrane showed yellowish to light brown plaques with a hazy gray infiltration in the surrounding area. The embryos were alive.

Microscopically, the membrane had developed a severe reac-

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DESCRIPTION OF PLATES

PLATE 23

- FIG. 1. Parasitic type of *Malassezia furfur* in periphery of inoculum (5 days). $\times 350$.
- FIG. 2. Section through membrane showing *Pityrosporum ovale* on, and invading, the ectoderm (5 days). $\times 430$.
- FIG. 3. Chorio-allantoic membrane infected with *Trichophyton gypsum*. Egg incubated 10 days and observed 7 days after inoculation.
- FIG. 4. Small spores and fine filaments in peripheral growth of *Trichophyton gypsum* (8 days). $\times 330$.
- FIG. 5. *Epidermophyton inguinale*. Cornified ectoderm with invading fungous filaments (8 days). $\times 330$.
- FIG. 6. Parasitic type of filaments of *Epidermophyton inguinale* as seen in cornified material on ectoderm (8 days). $\times 390$.
- FIG. 7. Parasitic filaments of *Achorion Schoenleini* in ectodermal detritus (5 days). $\times 430$.
- FIG. 8. Section through membrane inoculated with *Achorion Schoenleini*. Note gas bubbles in ectoderm (5 days). $\times 135$.

creased also by the invasiveness, in some cases, of the fungous elements.

The fungi grew luxuriantly on the chorio-allantois, were easily demonstrated with methylene blue and eosin, and in most cases showed a reversion to their parasitic morphology in from 5 to 11 days. Strangely enough, with yeastlike organisms this reversion may take place throughout the entire growth or, as is the case with some of the filamentous forms, at the periphery of the fungous inoculum, in the ectodermal layer or at the surface of the ectoderm where the amount of mycelium is relatively small.

By this method it has been possible to develop lesions, some of which have hitherto required human subjects. The value of the chorio-allantois for this purpose may be emphasized also because the cost is much less than if experimental laboratory animals are used and the time necessary for the development of diagnostic features frequently is much shorter. The gratifying results which have been obtained indicate great possibilities in investigative work with fungi and thus warrant the continued use of this method in mycology.

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PLATE 24

- FIG. 9. *Microsporium canis*. Fungous elements in translucent, granular exudate. Note marked ectodermal proliferation (5 days). $\times 95$.
- FIG. 10. Section through peripheral growth showing parasitic, budding cells of *Monilia albicans*, with monocytes and some leukocytes (6 days). $\times 800$.
- FIG. 11. *Monilia albicans*. Section through fungous growth and part of membrane. Note marked growth at periphery (6 days). $\times 80$.
- FIG. 12. Section through membrane inoculated with *Monilia albicans*. Note whorls of ectodermal cells and marked pearl formation in mesoderm (6 days). $\times 85$.
- FIG. 13. Ectodermal pearls in membranes infected with *Monilia albicans*, higher magnification (6 days). $\times 310$.
- FIG. 14. Parasitic cells of *Geotrichum versiforme* associated with inflammatory cells (5 days). $\times 350$.
- FIG. 15. *Geotrichum versiforme*. Section through fungous inoculum and portion of membrane. Note broken ectoderm and irregular fungous growth (5 days). $\times 80$.
- FIG. 16. Section through membrane invaded by *Zymonema dermatitidis*, showing the formation of spherical cells (5 days). $\times 440$.
- FIG. 17. Budding, thick-walled, yeastlike, parasitic cells of *Zymonema dermatitidis* in nodule formation on membrane (10 days). $\times 745$.

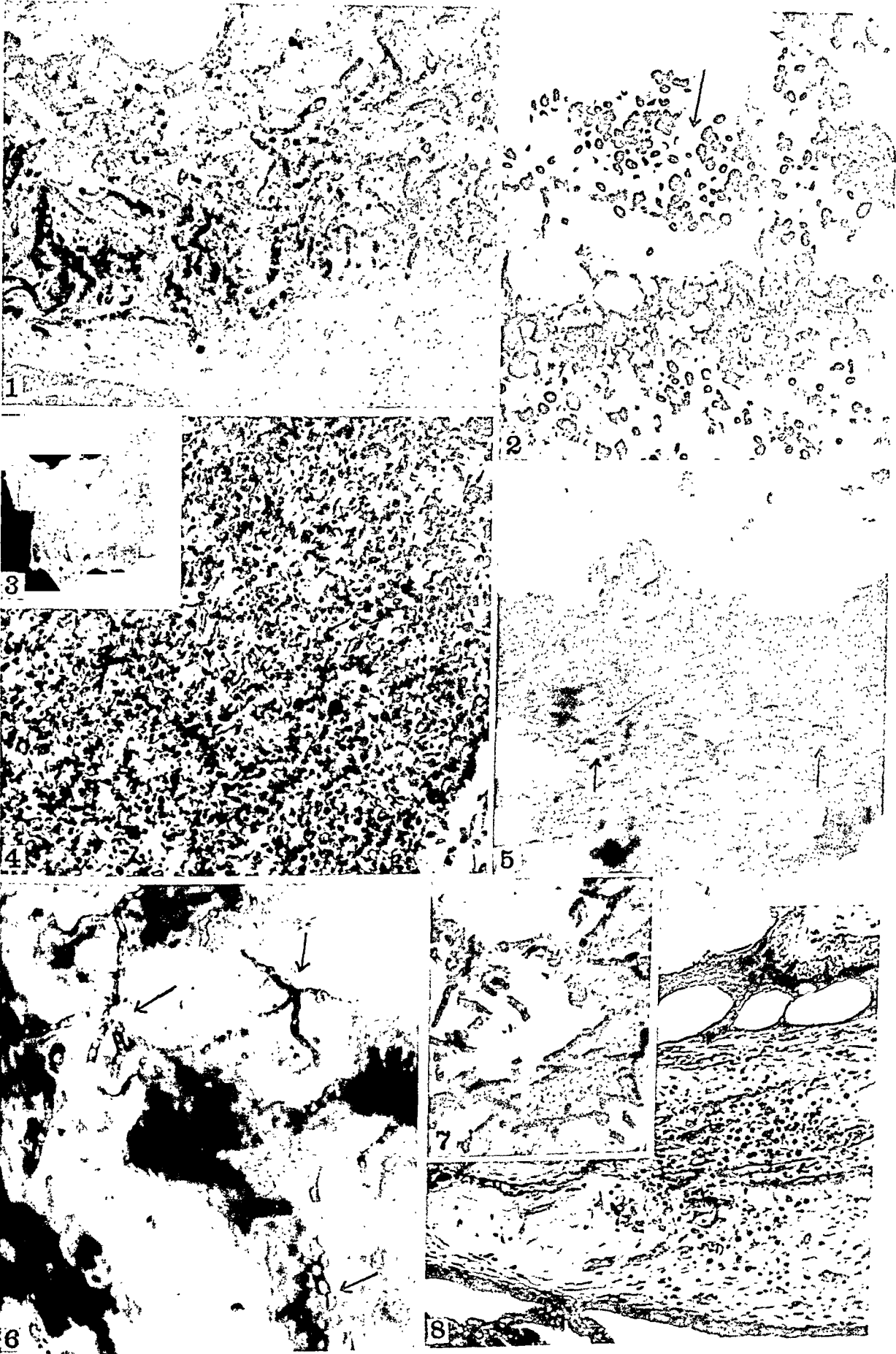
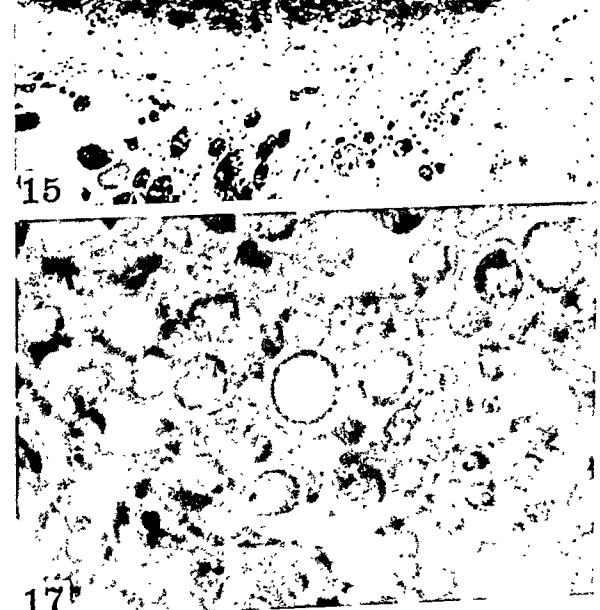
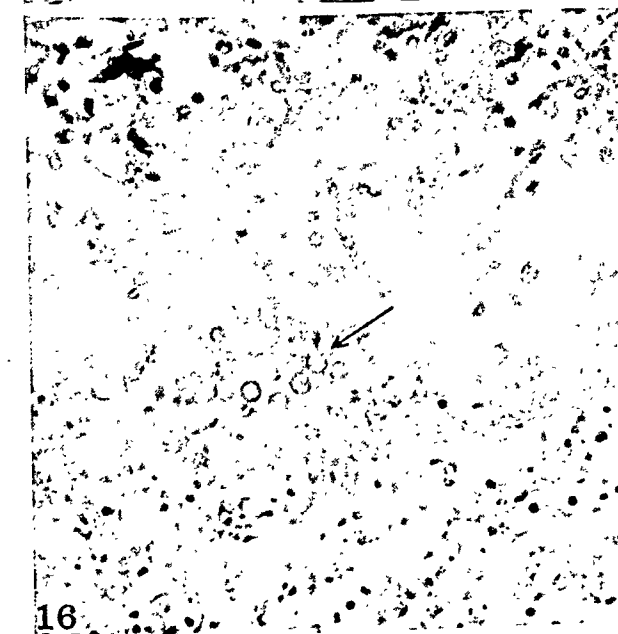
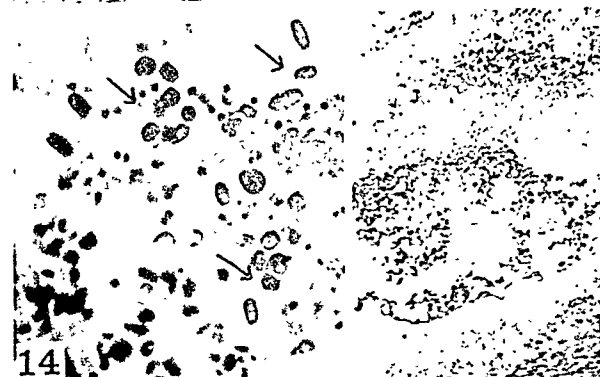
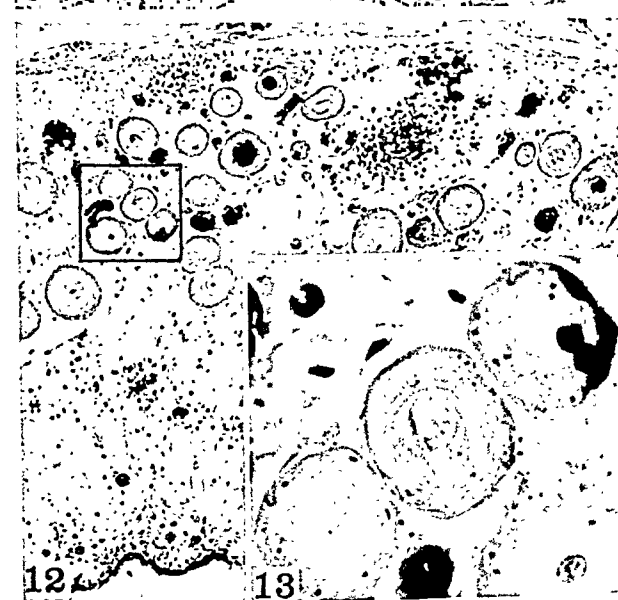
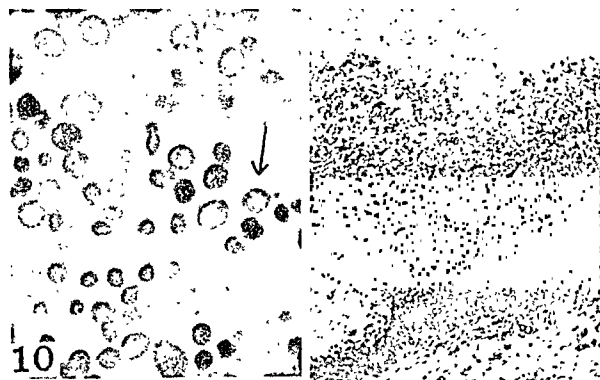
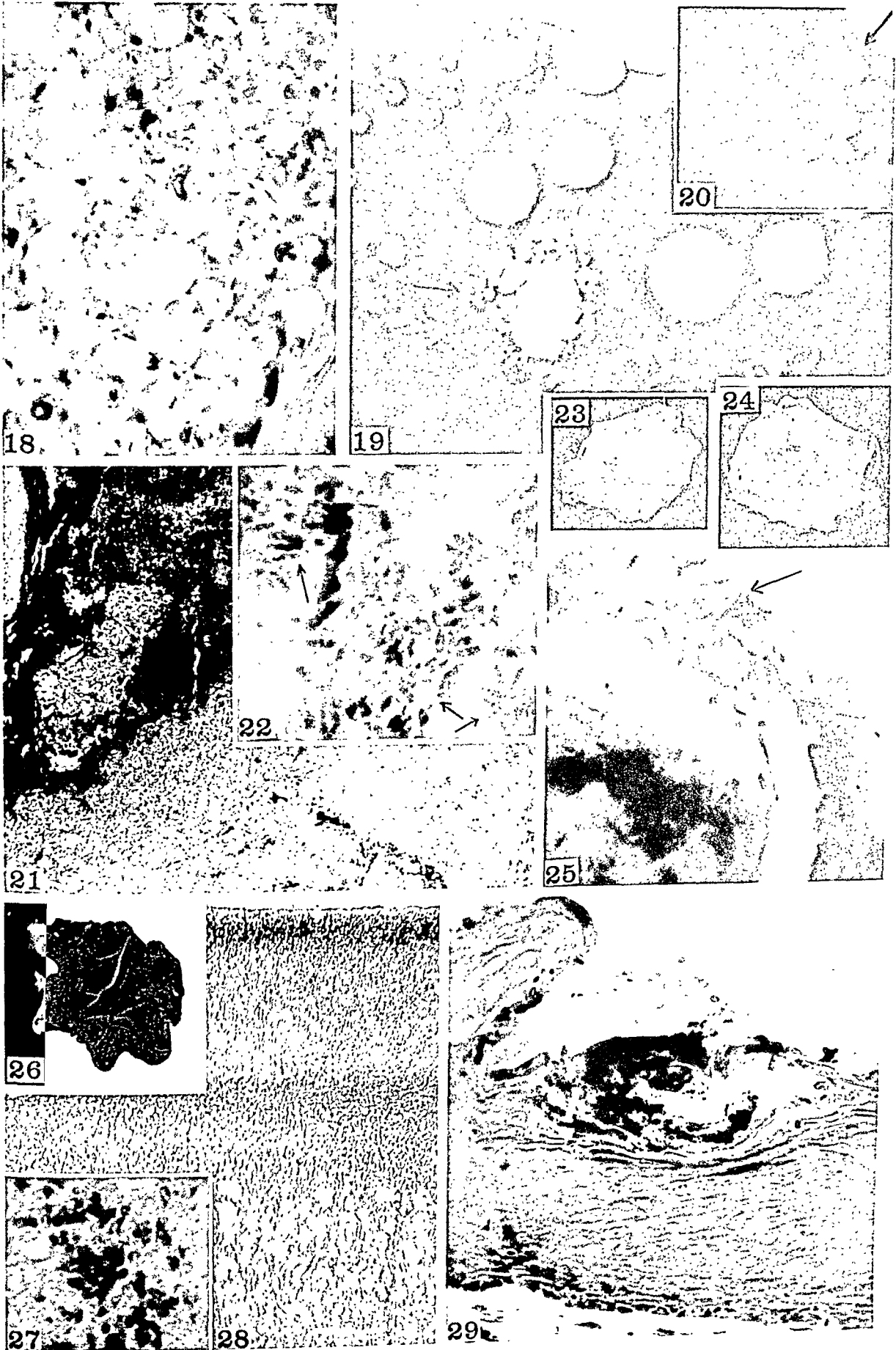


PLATE 25

- FIG. 18. Mucoid-encapsulated, budding, parasitic cells of *Cryptococcus hominis* on membrane (5 days). $\times 605$.
- FIG. 19. *Coccidioides immitis*. Transformation of hyphal cells to endospore-forming structures as seen in exudate (7 days). $\times 445$.
- FIG. 20. Endospore formation of *Coccidioides immitis*, partly hidden by overlying section, embedded in translucent, granular exudate (7 days). $\times 430$.
- FIG. 21. Section through inoculum and portion of membrane. Note translucent, granular exudate within which are seen the fine filaments of *Sporotrichum Schencki*. Note also reaction on ectoderm (10 days). $\times 85$.
- FIG. 22. Section of periphery of inoculum showing typical, cigar-shaped, parasitic cells of *Sporotrichum Schencki* occurring in clusters (10 days). $\times 880$.
- FIG. 23. Membrane infected with *Actinomyces bicolor*. Egg incubated 10 days and observed 7 days after inoculation.
- FIG. 24. Membrane infected with *Actinomyces bovis*. Egg incubated 10 days and observed 7 days after inoculation.
- FIG. 25. Filaments and spores of *Actinomyces bicolor* in ectoderm (8 days). $\times 1080$.
- FIG. 26. Membrane infected with *Monosporium apiospermum*. Egg incubated 10 days and observed 7 days after inoculation.
- FIG. 27. Conidia of *Monosporium apiospermum* in the membrane (5 days). $\times 415$.
- FIG. 28. Section through membrane showing zone formation and complete invasion by *Monosporium apiospermum* (5 days). $\times 80$.
- FIG. 29. Ectodermal proliferation as a result of presence of *Phialophora verrucosa* (8 days). $\times 305$.





ventricle, interventricular septum with left auricle and wall of right ventricle and auricle. The sections were stained with hematoxylin and eosin. Microscopical study showed that in addition to the large macroscopically visible focus in the wall of the left ventricle, there were numerous smaller areas of the same spongy tissue in other parts of the heart including the interventricular septum, right ventricular wall, right and left auricular walls and various papillary muscles. A subendocardial location of these foci was noted rather frequently, causing a slight to moderate local bulging of the wall into the ventricular cavities. The nodules composed of muscular tissue with large vacuoles were indistinctly outlined from the normal myocardial tissue and seemed to merge with it in many areas of their circumference. Normal myocardial muscle bundles and strands of vesicular, swollen, primitive; immature muscle cells were found interlocked in such regions.

The pathological tissue was composed of a spongy network in which more or less wavy, delicate fibrils surrounded huge, irregularly round, polygonal or oblong vacuoles, producing thereby the picture of a tissue consisting of an accumulation of partly collapsed and more or less empty bags. The majority of the vacuolar spaces were empty after having been subjected to the various procedures of fixation, dehydration and staining, but an appreciable number of them displayed some type of content. In most, a chromatic, elongated nucleus was present, apparently flattened against the wall of the vacuole. In other cells there was a cytoplasmic marginal rim containing a somewhat larger, irregularly round nucleus projecting into the vacuolar center (Fig. 1). Some cells contained a pinkish granular material, the granules showing sometimes an abortive striated arrangement. Similar granular or homogeneous cytoplasmic matter was seen to surround a centrally located round nucleus from which ribbon-like or fibrillar cytoplasmic processes extended to the cellular periphery ("spider cells"). Scattered throughout this primitive, embryonic myocardial tissue there were cells containing fragments of cross striated sarcous material (Fig. 2), while some of the large cells had a foamy cytoplasm with hyaline, blotchy inclusions, staining dark pink. Small groups of cells stained dirty bluish gray were observed in several places within the spongy areas, evidently representing calcified, degenerated primitive myocardial cells. Staining

RHABDOMYOMATOSIS OF THE HEART IN A GUINEA PIG *

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It is only in recent years that congenital rhabdomyomas of the heart have been reported, not only in the white race—48 cases; but also in other races—Negro, 1 case, and Japanese, 2 cases (Hueper;¹ Mitani;² Tamura³). The observation of rhabdomyomatosis of the heart in a guinea pig extends the occurrence of these rare blastomatoid formations of the cardiac muscle to a second species.

The present observation was the result of an incidental finding in a guinea pig used in an experimental investigation of the morphological action of digitalis glycosides upon the heart muscle. The animal died 4 days after the intramuscular injection of a digitalis preparation. Its weight was 245 gm. No information was available regarding its age. When examined *post mortem* the lungs were found to be congested and spotted with small dark red hemorrhagic areas. The left ventricle of the heart was firmly contracted, while the right ventricle was dilated. The heart measured 2 by 1 cm. On longitudinal sectioning it exhibited an irregular, indistinctly outlined, pale whitish red area measuring 0.5 by 0.75 cm. located in the apical portion of the left ventricular wall. The other organs were normal. The brain was not removed.

The lung, heart, liver, stomach, spleen, pancreas, suprarenal, kidney, testis and epididymis were examined histologically. Inasmuch as the pathological lesions noted were unrelated to the condition observed in the heart, it will suffice to list briefly the *histological diagnoses made*: purulent bronchitis, purulent pneumonia, marked congestion and edema of the lung; mild pericentral fatty infiltration of the liver; hemosiderosis of the spleen; moderate degeneration of the spermatogenic epithelium of the testis with accumulation of immature and partly degenerated spermatogenic cells within the epididymic ducts.

Sections from the heart were prepared from the wall of the left

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DESCRIPTION OF PLATE

PLATE 26

FIG. 1. Rhabdomyomatous tissue embedded in normal myocardium, showing the typical large vacuolar structure. $\times 230$.

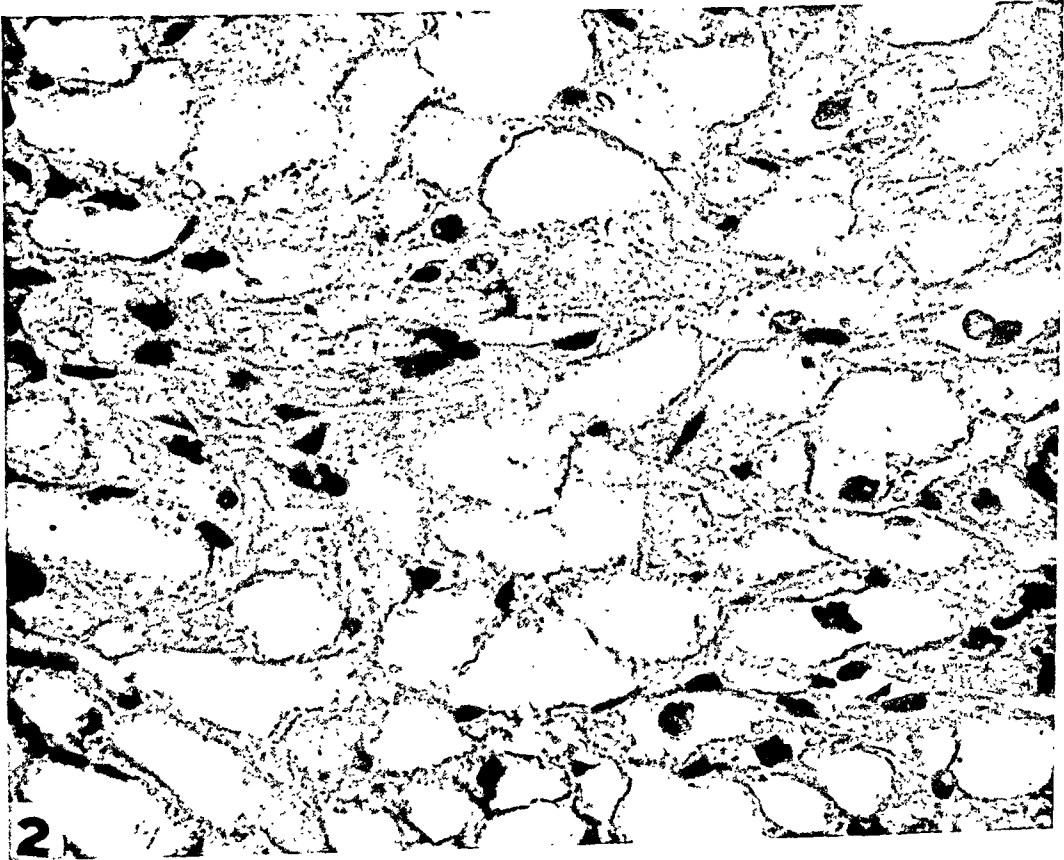
FIG. 2. Rhabdomyomatous tissue with primitive myocardial cells containing fragments of cross striated sarcous material. $\times 625$.

for glycogen was negative, but the heart had been for 1 week in a solution of formaldehyde before dehydration was started.

COMMENT

Inasmuch as the morphology of the spongy tissue areas in this heart duplicates exactly that seen in rhabdomyomas of the human heart, there can be no doubt that this is a case of rhabdomyomatosis of the heart of a guinea pig. The evidence presented supports the conception that rhabdomyomatous formations of the heart are not true tumors, but congenital tissue malformations with blastomatoid characteristics.

In a recent communication on von Gierke's glycogen-storage disease Humphreys and Kato⁴ raised the question whether several of the examples reported as local or diffuse rhabdomyomatosis of the heart might not represent a myocardial variety of von Gierke's disease (Pompe⁵). It may be pointed out in this connection that the glycogen contained in the primitive muscle cells of rhabdomyomas apparently is very much more soluble in the ordinary fixatives than the glycogen stored in the various organ cells (liver, heart, etc.) in glycogen-storage disease. This difference is brought out very strikingly by the fact that the demonstration of glycogen in the cells composing rhabdomyomas has been accomplished only very rarely, because the glycogen in this instance had been removed from these cells by fixation in aqueous media before the proper staining procedures were applied, while the glycogen present in the tissues of von Gierke's disease has been demonstrated by chemical and staining methods after having been in fixating fluids for weeks or months. This glycogen is not only markedly resistant to postmortem hydrolysis, but also very much less soluble in watery agents than ordinary glycogen, including that contained in rhabdomyoma cells. This histochemical criterion may help in the future, in addition to the demonstration of "spider cells" and various myofibrillar evolutionary manifestations in the primitive myocardial cells, in distinguishing between rhabdomyomatous lesions and myocardial tissue changes associated with von Gierke's glycogen-storage disease.



stomach. The wall of the entire stomach was thickened and there was a heavy muscle band at the pylorus such as occurs with hypertrophic pyloric stenosis.

The kidneys were congested and a small abscess was noted at the lower pole on the left side. No developmental disturbance was present.

Unfortunately the brain was not examined. The spinal cord showed congestion.

MICROSCOPIC EXAMINATION

The nodules in the heart consisted of groups of hypertrophied muscle fibers with large intracellular vacuoles. The nuclei were not destroyed but were usually found near the margins of the cells and were distorted in shape. Most of the nodules were definitely demarcated but occasionally the outer zone was less involved and approached the normal myocardium in appearance. The interstitial tissue was not increased and there was no inflammatory reaction.

Upon removal the heart was first placed in an aqueous solution of formaldehyde (4 per cent). After 2 hours a portion of the heart was transferred to absolute alcohol. Sections of the alcohol-fixed material were stained for glycogen by Best's method. Glycogen was present in the vacuoles in the large nodules but was absent in the minute nodules. It is possible that the glycogen dissolved out of the small nodules while in the formaldehyde, but the fat stains did not suggest this. Frozen sections of the formaldehyde-fixed material were stained with Sudan III. Fine droplets of fat appeared throughout the small nodules and at the periphery of the large ones. No large fat droplets were found. The fat was present in the involved muscle fibers but not in the large glycogen vacuoles.

There was hyperplasia of the muscle fibers in the stomach wall with a slight patchy hypertrophy of the individual muscle fibers. Sections of the stomach wall were stained for fat and glycogen with negative results.

The kidneys showed an acute pyogenic process with one larger abscess as previously noted and multiple minute abscesses. There was no evidence of maldevelopment.

CONGENITAL NODULAR GLYCOGENIC DEGENERATION OF THE MYOCARDIUM *

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Over fifty examples of so-called rhabdomyomatosis or congenital rhabdomyoma of the heart have appeared in medical literature, beginning with that described by von Recklinghausen¹ in 1862. The most recent summary is that of Labate² in 1939, which includes a bibliography and table of the significant findings. We present an additional case.

REPORT OF CASE

Clinical History. A white male infant weighing 8 pounds, 1 ounce was born uneventfully on June 20, 1940. It was the third sibling, with the first two living and well. During the first 10 days of life there was a mild diarrhea. This did not recur.

On July 28 the baby was readmitted to the hospital because of persistent vomiting after each feeding. He was dehydrated and his weight had dropped to 6 pounds. In spite of the administration of fluids and stimulants the infant died on July 29, the 40th day of life. The temperature rose to 109.4° F. before death.

POSTMORTEM EXAMINATION

An autopsy was performed 2 hours after death. The body was that of an emaciated, dehydrated white male infant weighing 3,100 gm. and measuring 54 cm. Externally there were no developmental disturbances.

The heart was normal in size but showed multiple nodules throughout the myocardium in both ventricles and auricles. These varied in size from those just visible to the naked eye up to 2 cm. in diameter. The larger nodules were found only in the ventricles. Some of the papillary muscles were involved. On cut surface the nodules were lighter in color than the normal muscle and were firm to the touch. This was the only cardiac disturbance found. The great vessels were normal.

The right lung had four lobes. The lower lobes of both lungs were atelectatic, probably due to pressure from the dilated

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rhabdomyoma or rhabdomyomatosis to a more suitable and distinctive term. *Congenital nodular glycogenic degeneration* of the myocardium agrees with the objective findings of the disease without suggesting a neoplastic origin or implying an exact knowledge of the etiology.

SUMMARY

A white male infant, dying on the 40th day of life, showed multiple nodules throughout the ventricular and auricular myocardium. The nodules consisted of hypertrophied muscle bundles with fatty degeneration and glycogen deposits in large vacuoles. Other anomalies present were a four-lobed right lung and hypertrophy of the entire wall of the stomach with pyloric stenosis. This condition has been erroneously termed congenital rhabdomyoma or rhabdomyomatosis. We suggest, as a more suitable name, *congenital nodular glycogenic degeneration* of the myocardium.

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DESCRIPTION OF PLATE

PLATE 27

FIG. 1. Section through the wall of the left ventricle. $\times 3.5$.

FIG. 2. Part of a nodule in the left ventricle. $\times 340$.

FIG. 3. The edge of a subendocardial nodule in a papillary muscle of the left ventricle. $\times 280$.

FIG. 4. A very small nodule in the wall of the left auricle. $\times 280$.

The spinal cord was congested and the anterior horn cells were undergoing an acute degeneration.

Summary. Dilatation of the stomach with generalized hypertrophy of the wall and pyloric stenosis. Acute purulent nephritis with gross and microscopic abscess formation. Multiple nodules in the auricular and ventricular myocardium containing glycogen and showing fatty degeneration (rhabdomyomatosis). Four-lobed right lung. Atelectasis of the lower lobes of both lungs. Acute degeneration of the anterior horn cells of the spinal cord. Marked emaciation and dehydration.

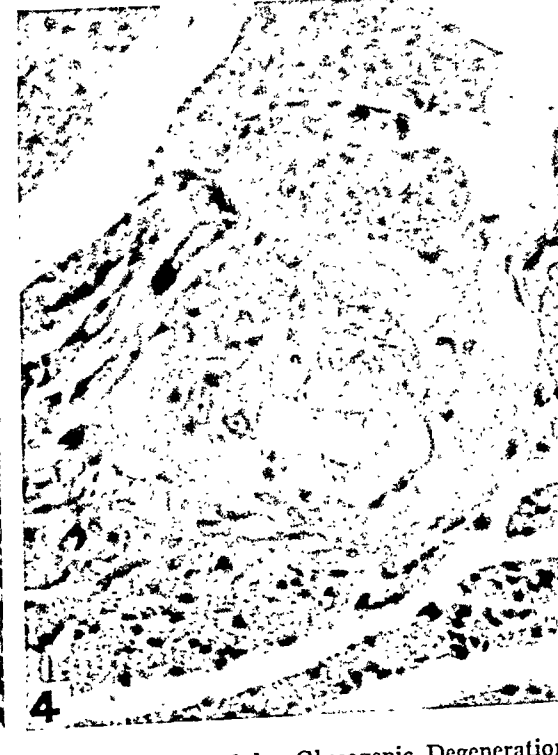
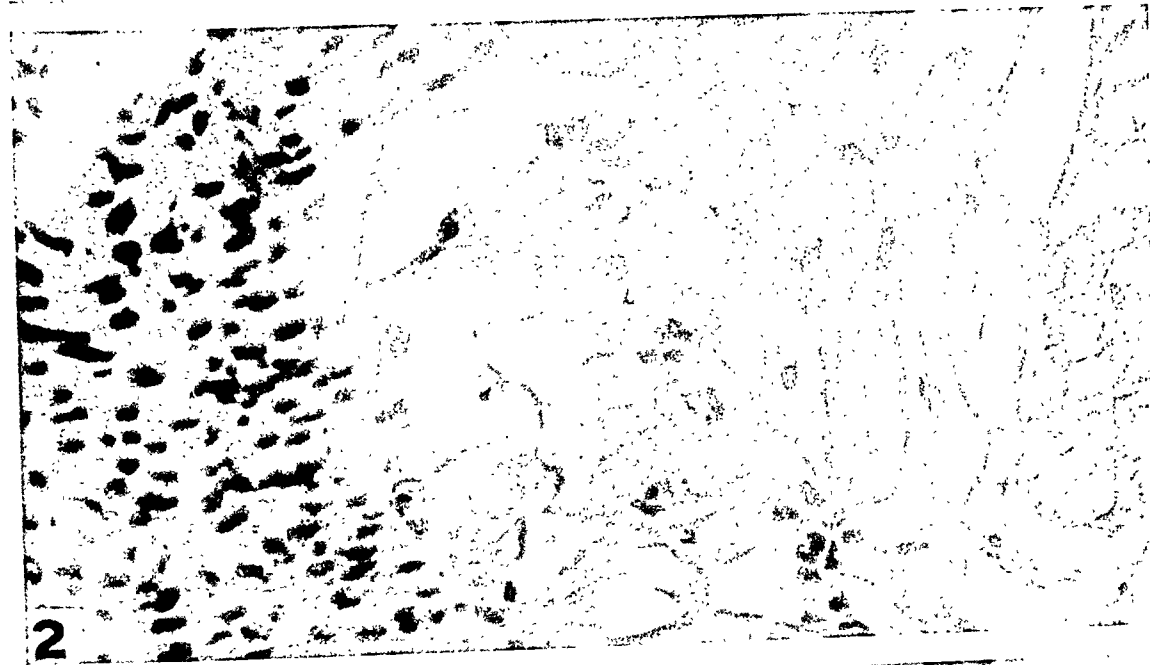
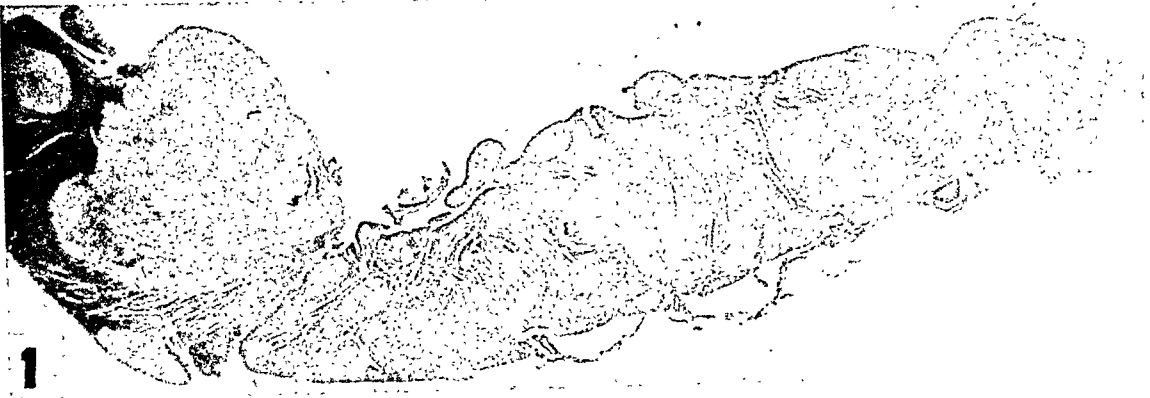
DISCUSSION

The findings in this case support the conclusion of Steinbiss³ that the myocardial nodules are a developmental disturbance with secondary degenerative changes. They are frequently associated with other anomalies, especially in the brain and kidney. Steinbiss concluded that the lesions are not neoplastic, and represent a local phase of a generalized condition rather than the mechanical type of malformation represented by tissue arrests. The individual lesions appear to be progressive and Steinbiss stated that they may, after degeneration, go on to scar tissue formation. There is no positive evidence that new lesions develop. The distribution of fat and glycogen in this case coincides with the description by Steinbiss but disagrees with that of Hueper⁴ who found no fat in the frozen sections of the heart in his case.

The cardiac lesions in this and many of the other cases in the literature are not directly responsible for death. Not more than one patient in ten reaches the age of puberty and 50 per cent die in the first year of life.

Almost without exception there is no mention made of the auricles in the recorded cases. In this infant both auricles showed nodules, most of which were of minute size. The uniformly small size of the auricular nodules supports the theory that there is no hyperplasia of muscle fibers but only hypertrophy of individual groups.

Because of poor terminology this condition has been confused with the rhabdomyoma, which is a true neoplasm. For this reason it seems desirable to alter the terminology from congenital



Nodular Glycogenic Degeneration

tic resorption of the subchondral bony plate, and in proliferation of blood vessels and fibrous tissue into the damaged articular cartilage. This vascularization of the damaged cartilage, which is followed by ossification, gives rise to the development of marginal exostoses that may be covered with cartilage, or may be without cartilage, as in those cases where the cartilage is worn away. The marginal exostoses are the most characteristic feature of osteo-arthritis deformans.

The functional theory of the development of osteo-arthritis deformans was developed by Beneke,² Pommer³ and Lang¹ after Nichols and Richardson⁴ had given a very exact description of the microscopic findings of this joint disease. The functional theory was confirmed by the studies of Allison and Ghormley,⁵ Parker, Keefer, Myers and Irwin,⁶ and others.

Almost all human joints have been subjected to microscopic study in order to find an explanation for the structural changes and the etiology of osteo-arthritis deformans except the temporomandibular joint. This joint has an exceptional place among the other joints due to its unique anatomical configuration and its more complicated function. The temporomandibular joint is a two compartment joint because of its division into a menisco-temporal and a menisco-condylar joint by the disk. However, all articular movements of the joint occur simultaneously in both compartments. The movements consist of rotary motion, antero-posterior gliding, lateral motion and circumduction. Circumduction is a combination of all the other movements and is the correct and ideal masticatory motion. Both left and right temporomandibular joints function simultaneously, one influencing the other.

It is important to remember that parts of the temporomandibular joint articulation are not developed at the time of birth. The articular tubercle is developed in the child as the result of the special function that it must serve. Furthermore, the normal relationship between the condyle and the mandibular fossa is determined and maintained by the harmonic balance of the complement of teeth, particularly by normal occlusion of the lateral teeth. The loss of lateral teeth produces a change in the location of the condyle. With the loss of all teeth, this interarticular relationship will eventually become more and more discordant. The

OSTEO-ARTHRITIS DEFORMANS OF THE TEMPOROMANDIBULAR JOINT *

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The contradictory opinions concerning the etiology and nature of osteo-arthritis deformans are responsible for the use of varied terms for this clinical entity that may involve any joint. The opinions of some authors, who base their conclusions on clinical observation and consider metabolic or endocrine disturbances, infectious disorders or senility as etiological factors in this joint disease, cannot be substantiated. A clear concept of this problem can be attained only through microscopic investigation of joints actually involved by osteo-arthritis deformans. Such studies evidence the fact that only two groups of arthritis can be distinguished; namely, an infectious inflammatory type and a chronic, proliferative, noninfectious inflammatory type.

Osteo-arthritis deformans is a chronic, proliferative, noninfectious inflammation which gradually leads to progressive mutilation of the joint. The inflammation is due chiefly to altered and impaired function of the joint. Further, this disturbance of the joint may be caused by injury of any type which decreases the elasticity of the articular cartilage, the main function of which is the protection of the subchondral bone against abnormal and damaging stresses. In other words, the cartilage serves as a cushioning mechanism. There may be mechanical trauma involving the joint directly or indirectly. Osteo-arthritis deformans can occur in young individuals (juvenile arthritis, Lang¹). It may also occur as the result of abnormally intensive use of the joint, as happens in certain occupations, or it may be produced by the wear and tear of advanced age. At any rate, the loss of the elasticity of the joint cartilage exposes the subchondral bone to constant mechanical injuries produced through impaired function. The bone marrow of the subchondral bone reacts to such prolonged traumata by inflammatory changes, resulting in osteoclas-

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destruction of the disk, were not associated with marked subjective symptoms.

NORMAL STRUCTURE OF THE ARTICULAR CARTILAGE AND THE DISK

In adults the articular surfaces of the condyle, the mandibular fossa and the articular tubercle are all normally covered by cartilage (Fig. 2), which consists of three distinct layers (Fig. 3). The innermost layer immediately adjacent to the subchondral bone is a zone of hyaline cartilage containing large basophilic cartilage cells. The second or middle layer is composed of a narrow strip of less basophilic cartilage cells arranged parallel to the surface of the bone. A dense accumulation of spindle-shaped cells separates the second layer from the third or outermost zone, which is wider and is made up of fibrocartilage, consisting of many fibers running parallel to the surface, and of a relatively few flat cells. This zone of fibrocartilage, resembling the cartilage of the clavicle and of the symphysis pubis, is covered by a very thin endothelium-like layer and, peripherally, there is a smooth transition into the stratum fibrosum of the periosteum and into the joint capsule (Fig. 2). This connection with the joint capsule is of great importance in solving the problem dealt with in this paper.

Relative to its cushioning function, the normal articular disk is made up of collagenous fibrous tissue arranged parallel to the surface of the condyle, and containing relatively few scattered chondroid cells grouped here and there. Elastic fibers are demonstrable between the collagenous fibers. Blood vessels are found in the central and peripheral portions of the disk. They assume a peculiar loop formation in the lateral or peripheral parts that are connected with the capsule (Fig. 2). This coil-like loop formation of the blood vessels indicates the compressibility of the disk; also its chondroid cells point to its mechanical function.

CHANGES IN THE ARTICULAR CARTILAGE

Pathological changes of the articular cartilage were found in almost all of the specimens that I examined, which included jaws of children with deciduous teeth, jaws of adults with a full com-

condyle of a normally functioning temporomandibular joint, in the closed position, is located adjacent to the posterior articular plane of the articular tubercle. This is the starting point from which all articular movements begin. Subsequent to the loss of lateral teeth, or more particularly to the loss of all teeth, the condyle, in closed position, is displaced posteriorly and superiorly in the mandibular fossa. It is obvious that such a change in the starting position of the condyle will be a decisive factor in guiding the condyle along improper surfaces in abnormal and unharmonious movements. These considerations indicate that disturbances of the balance of the complement of teeth may bring about pathological changes in the temporomandibular joint due to disordered function. That the temporomandibular joint articulation performs so many complicated movements makes it most satisfactory for the purpose of studying the traumatic alterations of the articular cartilage and their consequences.

In 1932 I⁷ published the results of a microscopic study of thirty-two temporomandibular joints obtained from cadavers ranging in age from 3 months to 68 years. These jaws varied from a full normal occlusion to complete edentulism. In most of them structural changes of the condyle, the disk, the articular tubercle and of the mandibular fossa were observed in varying degrees. These microscopic findings demonstrated that abnormal functional stresses, applied to the temporomandibular joint under certain anatomical or constitutional conditions, may lead to the characteristic changes of osteo-arthritis deformans (exostoses). Thus was made evident the importance of impaired function, *i.e.*, functional trauma or trauma of other origin, as the etiological factor in the development of osteo-arthritis deformans (Fig. 1). These findings were later confirmed by Steinhardt.⁸ Roentgenographic studies of the diseases of the temporomandibular joint have been conducted by Goodfriend,⁹ Riesner,¹⁰ and others.

I have since conducted microscopic studies on the temporomandibular joints of five additional cadavers. The findings are of significance because there was opportunity to examine the patients before death. It was possible to compare the clinical histories with the microscopic findings and thereby prove that, due to the accommodation of this joint to pathological function, morphological alterations, even to the degree of a complete

cartilage cells are flattened due to pressure, whereas other cells show degenerative changes of all degrees to complete vacuolar degeneration. In some cases these alterations involve the basal calcified layer, thus destroying the last protective barrier of the subchondral bone (Fig. 6).

These marked changes in the cartilage are associated with traumata of various kinds. The alterations of the cartilage, which involve the cartilage cells as well as their ground substance, are, according to Pommer,³ due particularly to edema, which is the result of the chronic traumata caused by impaired function. Further evidence of this concept has recently been presented in the studies of Callender and Kelser.¹¹ These authors described the presence of "blister" formations on the surface of the articular cartilage of human and animal joints and spoke of edematous or swollen cartilage. These "blisters" rupture and discharge a fluid into the joint cavity. The surface fringes of cartilage, common to this disease, are the remnants of these ruptured "blisters." Moreover, occasionally these fringes may result also from simple damaging stresses applied to the cartilage, with the same result as is seen in nicking a razor strop with a razor. In some of the cases of osteo-arthritis deformans which I studied, I found complete destruction of the cartilage layer with complete denudation of the subchondral bone, as has been described by Parker and co-workers.⁶ Occasionally, in regions where injury has been sustained over a long period of time, a diffuse calcification of the altered cartilage tissue occurs (Fig. 7). Such calcified areas, extending in some cases to the surface, induce ossification so that they become entrapped in bone. The surface of the articular cartilage assumes a hyaline appearance early. In a very advanced case of osteo-arthritis deformans of the temporomandibular joint in which the disk was destroyed, a portion of the condylar cartilage was necrotic.

Regenerative processes in cartilage can be seen as well as the degenerative changes described above. The most important finding in the damaged and inelastic cartilage is the vascularization which proceeds from the subchondral bone marrow. Vascularization (Fig. 8) is followed by ossification of the articular cartilage which is the phenomenon by which the characteristic exostoses are formed (Randwülste). The traumatic changes of the carti-

plement of teeth, and jaws of the toothless adult. The degree of alteration varied according to age and was much more intense in the cases where there had been a loss of lateral teeth or of all teeth.

In children the articular cartilage is strikingly different from that of adults. In children one does not find the calcified layer of cartilage or the subchondral bony plate. The trabeculae of the subchondral bone are arranged perpendicular to the articular surface of the condyle and the spaces of the bone marrow communicate directly with the articular cartilage, which is composed of fibrocartilage. This fibrocartilage consists of a layer of collagenous fibers which run radially from the surface of the subchondral bony trabeculae to the cartilage zone, where they turn tangentially and extend to the surface. This radially arranged layer contains relatively few cartilage cells.

The surface of the articular cartilage of the condyle, as well as that of the articular tubercle, of joints obtained from youths of 12, 17, 19 and 23 years of age shows a partially fringed appearance, whereas the inner portion is pierced by blood vessels which are accompanied by connective tissue advancing from the subchondral bone marrow. There are islands of bone surrounded by callus formation embedded in the cartilage.

Changes in the articular cartilage of the joints of older individuals are observed in the central areas of the articular surface and appear to be more severe. In addition to the development of fringes there are more or less extensive surface erosions. Associated with horizontal crevices of the superficial cartilage layer, it is frequently possible to observe vertical cracks starting on the surface and extending partly or even completely through the entire cartilage, and sometimes penetrating the subchondral bony plate. There are large horizontal fissures filled with blood which undermine the cartilage and separate it from the subchondral bone (Fig. 4). Furthermore, there may be complete destruction of the cartilage in localized areas, associated with an accumulation of broken down cartilage and callus-invested bone (Fig. 5). Occasionally this detritus forms a cyst which occupies the entire thickness of the cartilage layer. Vertical fibrillation of the cartilage can be seen extending through the calcified cartilage and the subchondral bony plate into the bone marrow. Superficial

with hemorrhages embedded in callus are found scattered in both the cartilage and the bone marrow spaces.

In addition to the degenerative changes of the cartilage, callus formation of young cartilage in connection with the traumatic interruption of the subchondral bony plate is present. This cartilaginous callus passes through the resorbed bony border and enters the marrow spaces (Figs. 10 and 11). In such an area I found a large cyst lined by connective tissue and partially surrounded by young cartilage cells with an island of cartilage cells in the periphery.

It is rational to assume that the islands of cartilage located in traumatically opened bone marrow spaces were displaced forcibly into them. However, the finding, in cases of extensive lesions, of islands of cartilage in distant marrow spaces containing normal fatty marrow has evoked many different explanations. According to Pommer³ and Lang,¹ these small, round islands of young cartilage cells, surrounded by very thin and small spindle-shaped cells (endothelial cells), which are found in unchanged bone marrow, were carried there by the blood or lymph as emboli. Of a contrary opinion were Parker and his co-workers⁶ who contended that these cartilage cells, at some distance from the joint line, were produced by the endosteum of the surrounding bone trabeculae or from the connective tissue of the marrow by means of metaplasia. Since it is a well established and generally accepted fact that cartilage is a product of grinding or rubbing stresses, I cannot bring myself to believe that bone marrow located so far away from the area of traumatic irritation could be exposed to such cartilage-inciting stresses, particularly since the normal fatty marrow remains unchanged.

EXOSTOSES

While the exostoses are generally believed to be the most significant finding in osteo-arthritis deformans, there is as yet no uniform opinion concerning their origin. According to Nichols and Richardson⁴ these characteristic bony protuberances are the result of perichondral or subchondral proliferation and develop by ossification of cartilaginous overgrowths. However, Pommer³ and Lang¹ proved, by their microscopic studies, that the exostoses arise from the subchondral bone marrow after vasculariza-

lage and the vascularization of cartilage from the opened marrow spaces can be observed in all articular planes, but more particularly in the peripheral regions. This striking finding is readily explainable because the cartilage at its periphery is intimately connected with the tissue of the joint capsule, as well as with that of the periosteum and therefore it is particularly exposed to tearing stresses that tend to detach it from the subchondral bone if the stresses exerted on the capsular tissue exceed the biologic limits.

CHANGES IN THE SUBCHONDRAL BONE

In the adult temporomandibular joints the borderline between the subchondral bony plate and the calcified articular cartilage is very irregular since there are areas of cartilage deeply embraced in the bone trabeculae. These findings point to an irregularity of ossification which can be explained by the fact that at the time of ossification, during youth, abnormal functional stresses disturb and inhibit uniform ossification.

Clefts, filled with blood, are sometimes observed perforating the subchondral bony plate and entering bone marrow spaces. Even in the absence of any changes of the covering cartilage this process is seen. In those cases where lesions of the cartilage had penetrated to the subchondral bone, resorption of the external surface of the subchondral bone by osteoclasts is observed and is also sometimes accompanied by osteoclastic resorption proceeding from the bone marrow spaces outward. Such bone marrow, however, is not of the normal fatty type but has been changed into fibrous tissue due to its reaction to irritative stresses (Fig. 6). In advanced lesions there are observed fractures of the subchondral bony plate with openings into the bone marrow despite the fact that in some cases the covering cartilage was partially maintained. I was able neither to observe any marked thickening of the original subchondral bone due to traumatic changes of the cartilage nor to see any necrosis of bone following fragmentation of the cartilage, as stated by Parker and co-workers.⁶

The fibrous bone marrow contains dilated blood vessels and it occasionally communicates with the altered cartilage (Figs. 6 and 9). Islands of bone produced by osteoclastic resorption along

cartilage. Cracks and fissures can be seen which are followed later by hyalinization of the disk tissue. One disk in particular shows extensive calcification of its dystrophic tissue while the disks of the joints with exostoses are either necrotic in those areas adjacent to the exostoses or are partially destroyed so that the joint becomes practically diskless.

SUMMARY

Forty-two human temporomandibular joints from individuals ranging from 3 months to 81 years of age were studied microscopically. Death had resulted from various diseases.

1. Most of the temporomandibular joints showed traumatic changes of varying degree. These changes primarily involved the cartilage of the condyle, the articular tubercle, the mandibular fossa and finally the disk structure.

2. The lesions were produced by impaired function through disturbed balance of the involved joint. The disturbed balance was due either to the loss of lateral teeth, loss of all teeth or to an external injury. All of these disturbances indirectly caused decrease or loss of elasticity of the cartilage, whose main function is the protection of the subchondral bone against abnormal functional stresses. This less elastic cartilaginous layer showed cracks, fissures and fringes. The cartilage cells underwent various types of degenerative changes such as fatty and mucoid degeneration, while on the other hand an overgrowth of the cartilage cells was observed. Also, dystrophic calcification of the cartilage, followed by ossification, was an occasional finding.

3. Involvement of the articular cartilage was occasionally followed by vascularization of the cartilage proceeding from the subchondral marrow after the subchondral bony plate had been resorbed. The normal fatty marrow of these opened spaces was transformed into a fibrous or gelatinous tissue containing dilated blood vessels, hemorrhages, areas of callus formation, accumulations of detritus and cartilage islands.

4. Vascularization and ossification of the cartilage layer produced exostoses that consisted of laminated bony trabeculae and contained cartilage cells which had escaped resorption. Marginal exostoses of the condyle occurred in areas where the cartilage was exposed to unfavorable stresses, and particularly, because of

tion of the damaged and inelastic articular cartilage. Despite the fact that this explanation, based on careful microscopic study, was confirmed by Erdheim,¹² Burckhardt¹³ and myself⁷ and more recently by Callender and Kelser,¹¹ there are other authors who still give a different explanation. Walter Bauer¹⁴ contended that this overgrowth originates as a result of the proliferation of the marginal area of the cartilage which later is transformed into true bone. Parker and co-workers⁶ believed that "flattening of the surface which is in contact with the opposing joint surface causes the edge of the bone to project outward as a shelf, and, depending on weight, pressure and position, the projection may curl or bend over at the edge."

In my opinion, the microscopic findings of this study give clear evidence that true exostoses originate from the subchondral bone marrow that first renders vascular and then ossifies the damaged cartilage. These exostoses develop not only in the peripheral cartilaginous area in which they overlap the bone, so that they take the appearance of a mushroom, but they also occur in the central portion of the articular plane, thus enlarging the condyle in its longitudinal axis. I could find no evidence to support the theory that the bone is forced outward by a central pressure. It is difficult to believe that such well calcified bone as the subchondral bone could be compressed centrally with curling and bulging of its periphery. In 1932 I⁷ described and illustrated the marked longitudinal enlargement of the condyle, clearly proving that the growth therein is uniform and cannot be accounted for by pressure activity. Such exostoses as shown in Figure 11 have highly polished surfaces and are partly covered by areas of necrotic articular disk. The periosteum does not directly participate in the formation of the marginal exostoses. However, osteophytes from periosteal bone formation may occasionally unite with these exostoses.

CHANGES IN THE DISK

Such pathological changes of the temporomandibular joint as have been described above must exert a damaging influence on the articular disk. The disks of the temporomandibular joints that I have studied reveal the same alterations as the articular

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DESCRIPTION OF PLATES

PLATE 28

FIG. 1. Pumice-like surface of a malformed condyle.

FIG. 2. A section from a normal temporomandibular joint, in close bite, of a woman 28 years old. Note the connection of the marginal articular cartilage with the tissue of the capsule and disk. $\times 4$.

FIG. 3. Normal cartilage of condyle separated by joint space from disk. $\times 96$.

its connection with the capsule tissue and the disk, when the equilibrium of the joint was disturbed. The periosteum did not actively participate in the formation of exostoses but deposits of incidentally formed periosteal bone may be united to the true exostoses. Furthermore, exostoses were formed in the central parts of the condyle, thus producing deformation in the longitudinal axis.

5. The disk showed all degenerative changes up to complete destruction, depending on the severity of the lesion.

6. Functional traumata of the cartilage of the temporomandibular joint promote the development of osteo-arthritis deformans.

7. Osteo-arthritis deformans is a chronic noninfectious proliferative inflammation.

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PLATE 29

FIG. 4. Cartilage of the condyle of a man 42 years old. Horizontal crack filled with blood and fragments of calcified cartilage and bone, over subchondral bone, the surface of which is undergoing resorption by osteoclasts. Adjacent bone marrow space is opened and its content has been transformed into fibrous tissue. $\times 72$.

FIG. 5. Traumatic interruption of subchondral bony plate of the mandibular fossa by osteoclastic resorption. Frayed cartilage showing cracks, callus formation, debris of bone and of calcified cartilage. $\times 67$.

FIG. 6. Condylar cartilage showing traumatic fissures. Calcified cartilage and subchondral bone are being resorbed. Bone marrow spaces close to surface contain fibrous tissue. $\times 68$.

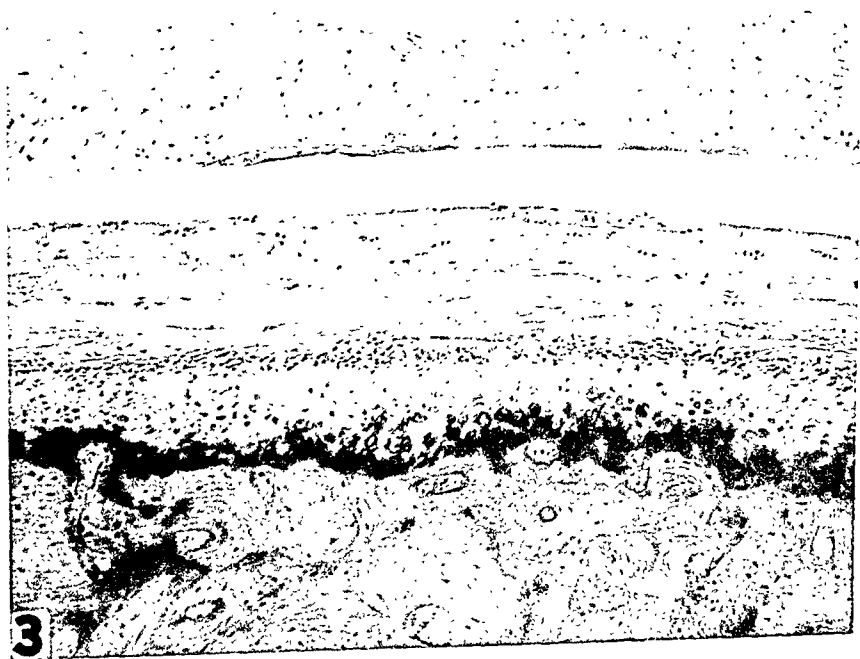
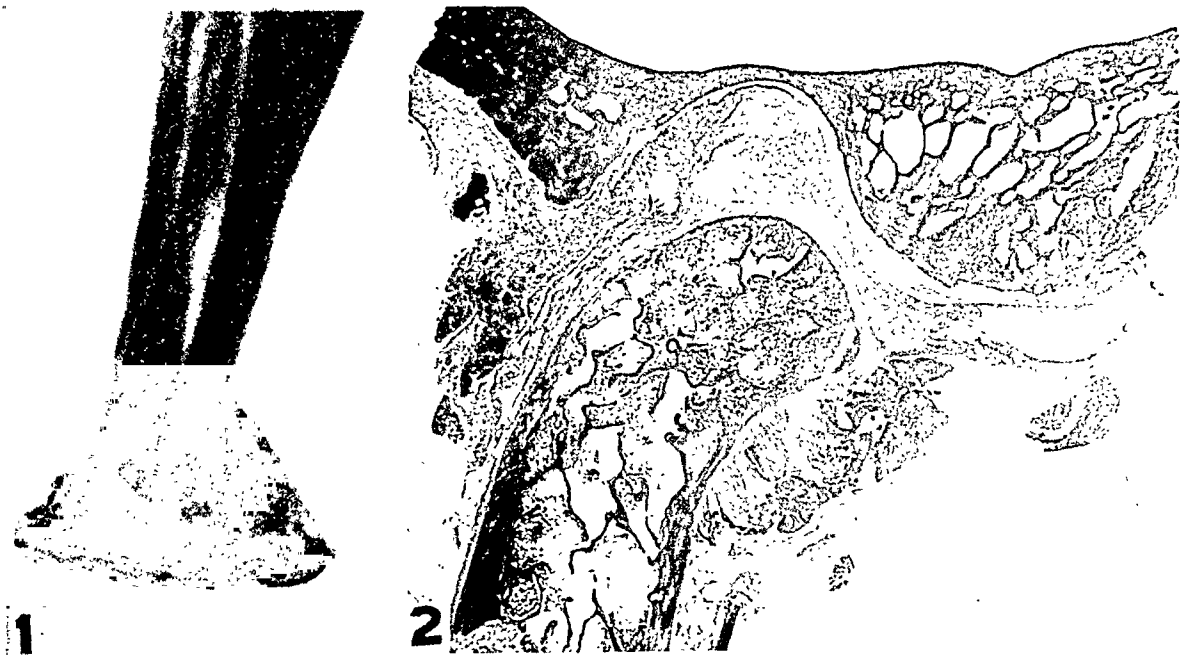


PLATE 30

FIG. 7. Calcification of damaged condylar cartilage followed by ossification. $\times 96$.

FIG. 8. Vascularization of the cartilage proceeding from opened bone marrow spaces. Necrotic fringes of the disk above the cartilage surface. $\times 125$.

FIG. 9. Widely opened bone marrow space on the surface of the subchondral bone of the condyle filled with edematous fibrous tissue, dilated blood vessels and hemorrhages. $\times 72$.

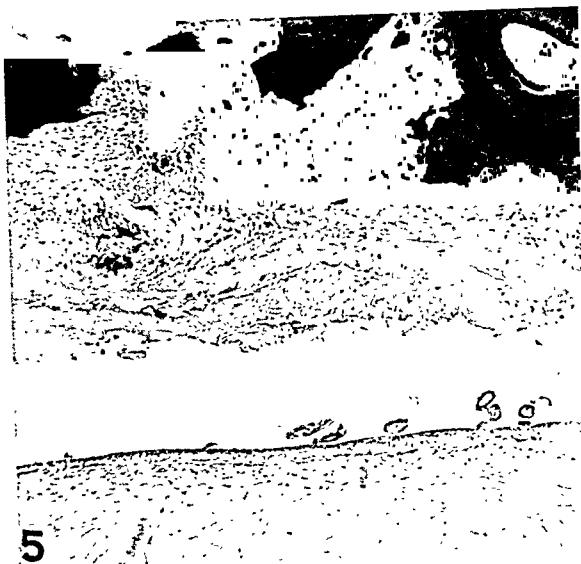
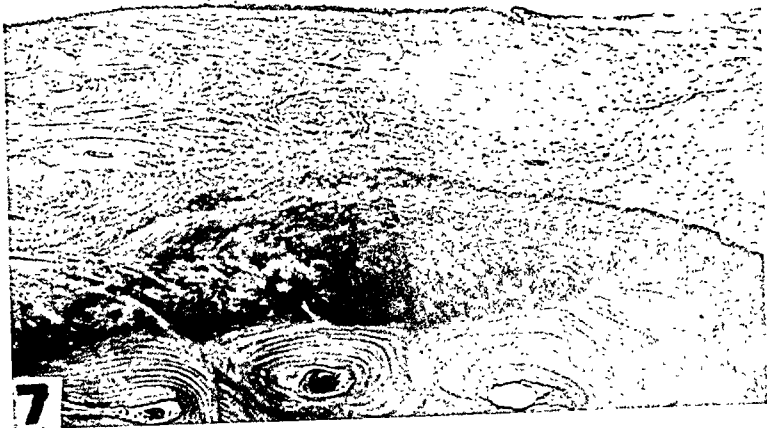


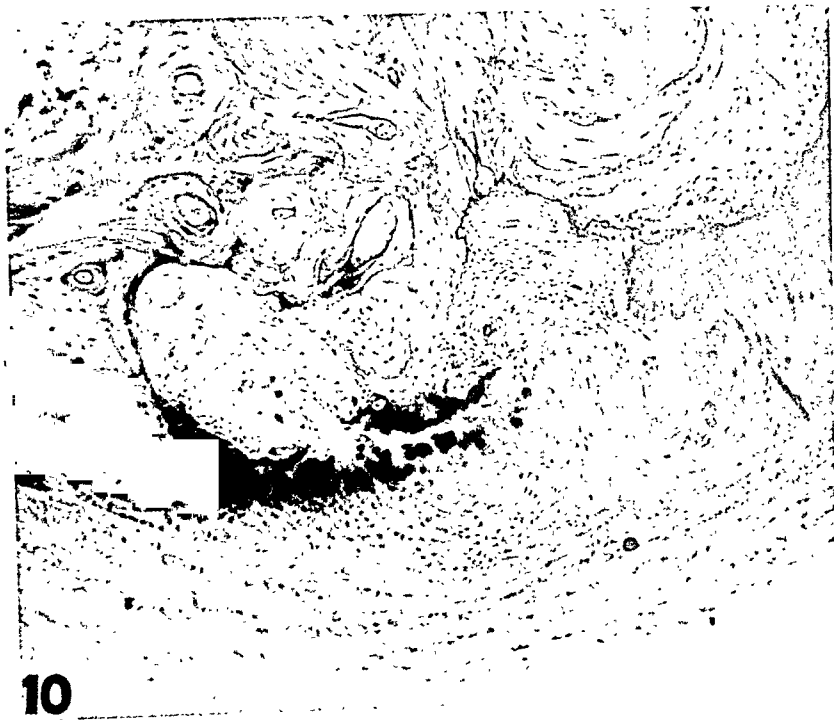
PLATE 31

FIG. 10. Traumatic interruption of subchondral bony plate of articular tubercle. Cartilage is invading fibrous bone marrow. The bony walls of the marrow spaces show osteoclastic resorption. $\times 67$.

FIG. 11. Resorption on the surface of the condylar subchondral bone with vascularization of the cartilage. There is a polished exostosis in the left corner. The surface of the cartilage is fringed and there are remnants of the necrotic disk. The bone marrow is fibrous. $\times 62$.



Temporomandibular Osteo-Arthritis Deformans



Bauer

Temporomandibular Osteo-Arthritis Deformans

grouped in a continuous layer extending for some distance in from the margins of the cells. The mature erythrocytes are, at this phase of the infection, relatively free of organisms but later on there is no longer any marked predilection for the reticulocytes. The morphology of the organism is by no means constant: on certain occasions coccoid forms predominate with but few rings; at other times the latter are present in great numbers. As the eperythrozoon diminish in number in the course of the infection, it may require some search to demonstrate true ring forms. While no exact blood studies have been undertaken, the notable increase in the reticulocytes suggests some degree of anemia. However, the infected animals are at no time obviously ill, nor is there pallor of the exposed skin of ears, feet and tail.

The infection produced in splenectomized white mice by the bartonella derived from the deer mouse is regularly severe and occasionally fatal. The organism appears in great numbers in the blood in from 3 to 8 days after inoculation and the infection soon reaches its height and subsequently runs a prolonged course characterized by remission and recrudescence. The initial rapid increase in bartonellae regularly terminates in a crisis characterized by great destruction of both red cells and microorganisms, the latter often not being microscopically demonstrable for a time. For several days following the crisis the animal is weak, somnolent, and there is staring of the fur together with extreme pallor of the exposed skin. However, no indication of hemoglobinuria has ever been observed. The fecal pellets are passed in long strings, owing to a great excess of intestinal mucus. The snipping of the tail to provide blood films is often followed by gangrene extending for a centimeter or more from the tip. Most deaths occur immediately following the onset of the initial crisis. Subsequent crises are in general less severe although death from bartonellosis has occurred 90 days after inoculation. Obviously death is due primarily to the sudden destruction of the red blood cells, the organs being pale as in an exsanguinated animal. The bartonellae at the height of infection occur in great numbers, some lying free but mostly distributed over the surface of the red cells. There is a sudden drop in body temperature at the onset of the blood crisis. Within 24 hours the rectal temperature may fall from 37.8°C. to below 32.5°C., the latter being the lowest reading that

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"INTERFERENCE" IN MIXED INFECTIONS OF *BARTONELLA* AND *EPERYTHROZON* IN MICE *

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In the study of a bartonella which occurs naturally as a latent infection in the local "white-footed" or "deer" mouse, *Peromyscus leucopus novaboracensis*, it was found that this species of bartonella was regularly infective for splenectomized white mice. It was then soon noted that the appearance of *Eperythrozoon coccoides* in the blood of the infected mice was promptly followed by the complete disappearance of the bartonella as judged by stained blood films. This observation led to a number of experiments to test the effects of these two infections one upon the other.

Preparatory to the consideration of the experimental data, it may be well to make certain comparisons in regard to the infections in question. *E. coccoides* is of frequent occurrence in common white mice and is commonly latent in laboratory stock mice, its presence being demonstrable by splenectomy. Following the removal of the spleen, the organisms appear promptly (3 or 4 days) and usually in great numbers in the circulating blood, adhering to the surfaces of the erythrocytes or free in the plasma. The reticulocytes, which occur normally in limited number in mouse blood, are somewhat increased and early in the infection have great numbers of the eperythrozoa adherent to them, often

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blood for eperythrozoa, were injected, 2 of them 11 days and one 10 days after splenectomy, with a suspension of blood of a deer mouse that had developed bartonellosis after splenectomy. Two of the splenectomized mice were injected with blood of a Peruvian mouse, *Phyllotis amico amico*. A blood examination several days later showed all 6 mice to be infected with *E. coccoides* irrespective of whether they received blood from *Peromyscus* or from *Phyllotis*. Hence there was reason to believe that all 6 mice of the series were infected with *E. coccoides* previous to splenectomy. Although all 6 were later again injected with mouse blood containing bartonellae in great numbers, no bartonellae appeared at any time in this group.

Group II. Splenectomy was performed on each of 11 white Swiss mice in batches on three successive days and, without preliminary blood examination, 2 to 4 days later they were injected with blood of the same deer mouse employed for group I. At the first observation, 4 days after inoculation, 5 showed bartonellae and 1 a mixed infection of eperythrozoon and bartonella. Nine days after inoculation the eperythrozoon had replaced the bartonella in this mouse, 1 had died of bartonellosis, and 9 were suffering in varying degrees from bartonellosis. On the following day eperythrozoa appeared in mixed infection in the bloods of 2 other mice and 6 days later the eperythrozoon had replaced the bartonella in all the surviving mice. As these animals were kept in two jars and were thrown together in the course of making blood films, the results suggested that one or more mice of this series were infected with *E. coccoides* before inoculation and that the infection spread gradually to all mice of the series.

Seventeen days after inoculation the 10 surviving mice were each treated by subcutaneous injection with 2.5 mg. of sulfarsphenamine, and in addition one half this amount was administered on the following day except to 1 mouse that died from the effects of the first injection. Sulfarsphenamine in adequate dosage has proved effective in eliminating both the eperythrozoon and the bartonella infections, and its injection in this instance resulted in the disappearance of the eperythrozoon from all the mice. Thirty-one days later the surviving mice were injected with blood from a mouse showing heavy bartonella infection. In 9 days 2 mice were dead of bartonellosis and 5 of the 7 survivors

could be obtained with the thermometer employed. Even with such very low temperatures recovery usually occurs, with readings gradually becoming higher so that, within a week of the onset, the temperature will have returned to normal.

It should be understood that *E. coccoides* occurs naturally in the common mouse, while the bartonella under consideration is derived from another host, the white-footed mouse. Many rodents carry latent bartonella infections, but none of the stock of white Swiss mice used has developed a bartonellosis on splenectomy.

EXPERIMENTAL

Bartonella infection was produced in each experimental animal by both subcutaneous and intraperitoneal injection of a suspension in citrated saline of the blood of either an infected *Peromyscus* or a common mouse. *E. coccoides* was always readily obtained from splenectomized stock mice and was similarly transmitted by the injection of the blood of an infected animal. The course of each infection was followed in the inoculated animal by the study at frequent intervals of blood films stained with Giemsa's stain and through the observation of such symptoms as occurred. Instruments employed in obtaining blood for films should always be sterilized between each operation. The bartonella of the deer mouse and *E. coccoides* are distinguished by their morphology and their distribution. The occurrence of rods and coccoids in chains and distributed quite generally over the surface of mature red cells has been regarded as satisfactory evidence of bartonellosis. The presence of rings in the plasma and on the margins of reticulocytes but never in chains has been considered sufficient for the diagnosis of eperythrozoon. Most forms of this organism are stained less intensely than the bartonella by Giemsa's method. While it would be quite impossible to identify individual coccoids, after gaining an acquaintance with morphology of the two organisms, no great difficulty is experienced in determining whether one or the other is present in appreciable numbers or both in mixed infection. The presence or absence of the two organisms at each observation, together with other data, is recorded in Table I.

Group I. Splenectomy was performed on each of 6 young white Swiss mice which, without preliminary examination of the

The results obtained in this group clearly show that eperythrozoon infection not only prevents the symptoms of bartonellosis but also prevents the multiplication of bartonellae in the blood. Under such circumstances, however, the bartonella may persist as a latent infection and later on it may gain the ascendancy when the eperythrozoon will then in turn become latent. Its presence, however, can be demonstrated by the injection of clean splenectomized mice. Thus a blood from which the eperythrozoon has long since disappeared microscopically may still be infective.

Group IV. For this series 7 mice were splenectomized and, having been proved to be free of eperythrozoa, 3 were injected with blood of an eperythrozoon-infected mouse. Ten days later these and the other 4 were injected with a suspension of pooled blood of 3 bartonella-infected mice. Bartonella was found on only one occasion in mixed infection with eperythrozoon in 1 of the eperythrozoon-infected mice, the observations having covered a period of 36 days from the inoculation of bartonella.

Group V. Eight recently weaned white Swiss mice were splenectomized, several on each of 3 consecutive days, and in from 2 to 4 days later were injected with heart's blood of mouse No. 9762, which had shown bartonellae 10 days previously but not when used for the present series. Since infection did not appear promptly in most of the inoculated mice, the entire group was reinoculated with heart's blood of mouse No. 9756, in which there were moderate numbers of bartonellae.

Ten days after the first inoculation all mice of the group were showing a heavy bartonella infection, and the first 4 of the group were inoculated with the blood of a mouse that had recently shown eperythrozoa, but this organism could not be demonstrated in a blood film taken at the time of inoculation. Since no eperythrozoon had appeared during the next 4 days, these 4 mice were again reinoculated with blood from a mouse showing moderate numbers of eperythrozoa. One animal, very weak from bartonellosis, died immediately. In the 3 eperythrozoon-treated mice, eperythrozoa appeared in considerable numbers 3 days after the last injection, but were greatly outnumbered by bartonellae. In the course of the following 5 days the bartonella was entirely replaced by the eperythrozoon.

were pale and extremely weak. Two (No. 9747 and No. 9751) showing the most severe symptoms were at this time injected with the blood of an eperythrozoon-infected mouse. In these the eperythrozoon promptly replaced the bartonella, but in 3 of the 5 other mice the bartonella also disappeared, to reappear long after in 1. Since it became apparent in this experiment that the bartonella could disappear spontaneously following the second crisis in a given animal, the conditions of this experiment were not suitable for demonstrating the effects of eperythrozoon infection on bartonellosis. The spread of eperythrozoon infection through the confinement of infected and noninfected mice in the same jar is suggested by the eventual appearance of this infection in mouse No. 9743, which was kept with the 2 eperythrozoon-inoculated mice.

Group III. Splenectomy was performed on each of 6 mice, and blood examinations made during the following month indicated that they were free of eperythrozoa. Three were then injected with blood containing *E. coccoides* and developed heavy infections. Fifteen days later all 6 were injected with *Peromyscus* blood showing great numbers of bartonellae. The 3 mice infected first with *E. coccoides* showed no bartonellae and presented a normal appearance over a period of 26 days, but on examination 56 days after the injection of bartonella, 2 were somewhat pale and presented a typical bartonellosis. This persisted for a period, but in both animals was again suppressed by a recurring eperythrozoon infection. At the height of the late-appearing bartonellosis, blood from each of the 3 eperythrozoon-treated mice, 2 showing at this time great numbers of bartonella, and 1 no organisms, was injected respectively into 3 mice, previously shown on splenectomy to be free of infection, in order to test for the presence of the eperythrozoon. Two of these developed eperythrozoon infection and 1 showed eperythrozoon and bartonella in mixed infection, the former organism soon replacing the latter.

The 3 untreated controls developed a severe bartonellosis and 12 days after inoculation were weak, pale, and showed staring of the fur, in marked contrast to the normal appearance of the mice inoculated with eperythrozoon 15 days prior to injection of the bartonella.

The dosage of eperythrozoa injected less than 2 days previous to the crisis of bartonellosis was evidently too small to cause an immediate heavy infection and the symptoms were quite as severe in the eperythrozoon-treated mice as in the controls. On recovery from the crisis both treated and untreated mice became sleek and normally active and there was nothing distinctive in the general appearance of the two series. However, the continuing bartonellosis in the untreated mice was in each case associated with pronounced anemia, while the blood of the eperythrozoon-inoculated mice rapidly improved to approximately normal.

The ease with which eperythrozoon may be unintentionally introduced is illustrated in the results obtained in 3 mice not included in Table I, which were injected with blood showing great numbers of bartonellae. Prior to their inoculation 2 mice previously infected with eperythrozoon were injected with the same material, and the precaution was not taken of changing the hypodermic needle before injecting the clean group. Six days later, one of the 3 mice presented large numbers of eperythrozoa, and 2 a mixed infection of eperythrozoon and bartonella.

DISCUSSION

While there are examples reported concerning the so-called "interference phenomenon" or "sparing effect" in regard to the influence of one infection upon another, the subject is one that appears to have received comparatively little attention. The results of the present study are of interest in that they are unusually clear-cut. The contrast between the appearance of groups of bartonella-infected mice at the time of the crisis of the infection and that of parallel groups infected with *E. coccoides* before being inoculated with the bartonella is very striking. While the former may appear so ill that recovery would seem improbable, the eperythrozoon-treated mice all maintain a normal appearance and remain sleek and normally active. The nature of the eperythrozoon and bartonella infections under consideration makes it possible to judge results on the basis not only of symptoms and pathological changes, but also on the course of the two specific infections in the blood as followed by periodic examinations of stained films. Observation is therefore more simple and

direct than in the case of virus diseases and most bacterial infections. The conditions are very similar to those which obtain in malarial infections, in which the organisms may be detected and their relative numbers estimated by microscopical examination of the blood. In fact, a similar effect in the tendency of one type of human malaria to exclude another has been repeatedly observed by malariologists.

The results obtained in malarial infections, however, do not appear to be as uniform as in the mixed bartonella-eperythrozoön infections. Thus James¹ (1931) found the incubation period of *Plasmodium falciparum* to be increased in a patient with *Plasmodium vivax* infection. On the other hand, both Antić² (1925) and Boyd and Kitchen³ (1937) found that the simultaneous infection of patients with *P. vivax* and *P. falciparum* is frequently followed by the early appearance of both species, but that the latter soon gains the ascendancy while the former may entirely disappear for a long period. Later on *P. vivax* may reappear and replace the other species. There is difficulty in the interpretation of such cases on account of their limited number and of the possibility of spontaneous remissions such as occur when only a single infection is present. Although it would be possible to collect a considerable number of references to the literature in regard to the effects of one infection upon another, it does not seem appropriate to review the entire subject until more is learned of the principles basic to the phenomena under discussion.

In regard to terminology, while "interference," "antagonism," and "sparing effect" have been applied more generally to the visible effects of infections rather than to the actual presence and multiplication of organisms or viruses, it is doubtful whether it would be desirable to introduce a new term for the phenomena under discussion, in infections in which fluctuations in the number of organisms may be followed by periodic examination of stained films. The term "interference" appears to be widely used and quite generally applicable.

In a previous paper (Tyzzer and Weinman,⁴ 1939) it was noted that with the great variety of blood infections found in the vole, *Microtus pennsylvanicus pennsylvanicus*, no mixed infections of bartonella and eperythrozoön were found. A series of splenectomized white mice was experimentally infected with the

Group	Mouse No.	Splene- ctomy	Epery- throzoon inoculation	Barton- ella inoculation	Dates of observations																								
					March					April					May					June					July				
					4	11	21	30		4	8	15	17	18	22	29	1	2	3	4	6	1	10	19	25	5	11	12	23
III	9730	Feb. 20	Mar. 26	Apr. 10	O	O	O	O		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9731	" "	" "	" "	O	O	O	O		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9733	" "	" "	" "	O	O	O	O		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9735	" "	None	" "	O	O	O	O		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9737	" "	" "	" "	O	O	O	O		O	O	O	O	O	B	B	B	B	B	B	B	B	B	B	B	B	†		
IV	9758	Apr. 25	May 4	May 14	April 29	O	O	O	O		O	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9759	" "	" "	" "	O	O	O	O		O	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9763	" 29	" "	" "	O	O	O	O		O	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9756	" 25	None	" "	O	O	O	O		O	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
	9757	" "	" "	" "	O	O	O	O		O	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	†		
V	9761	" 29	" "	" "	O	O	O	O		O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9762	" "	" "	" "	O	O	O	O		O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9781	July 1	July 15 and 19	July 5 and 12	July 5	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9782	" "	Ibid.	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9783	" "	Ibid.	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9784	" 2	Ibid.	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9785	" "	None	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9786	" 3	" "	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9787	" "	" "	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		
	9788	" "	" "	Ibid.	Ibid.	O	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	†		

E = eperythrozoon; B = bartonella; (B) = degenerating bartonella; O = no organism found; s = sulfarsphenamine injected.
 * = kept in jar with mice No. 9747 and No. 9751; inoculated May 13 with eperythrozoon.
 † = killed.
 ‡ = died of bartonellosis.

(In mixed infection precedence is given the preponderating organism.)

the type of interference in which one infection prevents or replaces another.

While the existence of eperythrozoon or of bartonella infection in experimental animals may be ascertained by splenectomy, it is important to observe certain precautions in order to prevent accidental transmission when employing groups of animals which are confined in a single cage or jar. Thus the snipping of the tails of mice or rats in the preparation of blood films may lead to the smearing of the surroundings and often of the fur or skin of another animal, which may then lick off the infective material. Carelessness in regard to the sterilization of the hypodermic needle when inoculating series of animals, or of instruments used in obtaining blood for films, or in punching or otherwise marking the ears, may result in accidental infection.

The practical bearing of the phenomena under discussion lies in the potentiality of unrecognized infections on occasion to reverse experimental results. An interesting field is opened in testing the possibilities of effecting protection against serious infections by vaccinating with harmless ones.

SUMMARY

A bartonella occurring naturally in the local "white-footed" mouse *Peromyscus leucopus novaboracensis* is pathogenic for splenectomized normal white mice, producing a severe and occasionally fatal anemia.

E. coccoides infection occurring naturally or induced in splenectomized white mice prior to their inoculation with the *Peromyscus* bartonella interferes with the development of the latter and prevents all symptoms. In such animals the bartonella, although not microscopically demonstrable, persists latently and may, after many weeks, replace the eperythrozoon infection which in turn becomes latent.

E. coccoides, introduced after the bartonellosis is established, promptly suppresses the latter infection so that bartonellae are no longer demonstrable in stained blood films.

The eperythrozoon of the white mouse has developed in a certain proportion of the splenectomized voles (*Microtus pennsylvanicus pennsylvanicus*) inoculated, but in this foreign host does

vole bartonella but certain failures subsequently noted in the inoculated mice have since been found to have occurred in individuals that were carrying *E. coccoides* infection.

Preliminary experiments have shown that it is possible in some instances to infect splenectomized voles with *E. coccoides* of the common mouse. Two of four old voles inoculated showed infection, which in one was light and transient, in the other quite heavy and prolonged. On being injected 15 days later with *Haemobartonella microti*, both developed a well marked bartonellosis. Thus *E. coccoides* in an alien host has failed to interfere with the development of a bartonella occurring naturally in that host.

No serological basis is known for the protective action of pre-existent *E. coccoides* infection against the *Peromyscus* bartonella in the tame white mouse, nor for the suppressive action of the former when introduced in established infections of the latter. The uncomplicated bartonellosis continues for many weeks with an abundance of organisms in the blood, so that the sudden microscopic disappearance of the bartonella attending the increase in the eperythrozoa is difficult to explain. The presence in the blood of considerable numbers of eperythrozoa is evidently essential in order to replace the bartonellae. Furthermore, a bartonellosis with great numbers of organisms in the blood may develop after the eperythrozoon infection has become latent, though still demonstrable by transfer to clean splenectomized mice. Long-standing bartonella infection affords no protection against eperythrozoon infection, as shown by a number of test animals. Such animals cannot be reinfected, however, by reinoculation with bartonella.

While elevation of body temperature is regarded as important in the treatment of syphilis involving the central nervous system by induced malaria, such is not the case in the suppression of the bartonella by the eperythrozoon. The temperatures taken in eperythrozoon-treated and untreated mice with bartonellosis from before the crisis up to their symptomatic recovery averaged slightly lower in the treated mice even during the period of the disappearance of the bartonella.

It is quite possible that "chemotherapeutic interference," as reviewed by Findlay⁵ (1939), may in some way be related to

not interfere with the development of severe bartonellosis due to the vole bartonella.

The possibility of the reversal of experimental results through the presence of unrecognized infection is pointed out, and that there may be a possible utilization of the principles of interference in the field of vaccination is suggested.*

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* Subsequent to submitting this paper for publication, additional observations were made on eperythrozoon and bartonella infections occurring naturally in the gray-backed deer mouse, *P. maculatus gracilis*. An eperythrozoon infection, becoming apparent in two animals after splenectomy, kept in suppression a bartonella infection for a period of about 6 weeks, at the end of which the latter organism gained the ascendancy in both animals. Thus "interference" is effective following splenectomy in eperythrozoon and bartonella infections coexisting in their natural host.

there are no reported studies on the effect of sensitization upon the development of ocular tuberculosis produced by intravenous injection of relatively small doses of living tubercle bacilli.

E. L. Opie and his associates, to whom we are indebted for this material, have for many years been studying experimental tuberculosis in rabbits with special emphasis on the relationship of sensitization to immunization. As complete autopsies were performed on all control or immunized rabbits that died or were killed during the course of these experiments, it seemed probable that a careful anatomical study of the eyes might give some insight into the pathogenesis of experimental ocular tuberculosis. An opportunity would also be afforded to compare the development and progress of ocular tuberculosis in normal and in immunized rabbits and its relationship to systemic tuberculosis and especially to tuberculosis of the lungs.

METHOD

The preparation of the vaccine, the method of immunization and infection of the animals have been described in detail by Opie and Freund.¹²

The infecting dose was a suspension of tubercle bacilli diluted so that 0.5 cc. contained 0.00001 mg. The content of each tube was stirred vigorously in order to break up, as far as possible, any clumps which might remain. The material was injected into a marginal ear vein. Amounts of suspension equivalent to 0.00001 and 0.000001 mg. were cultured on slants of Petragnani's media. There was considerable variation in the number of viable organisms; however, in the majority of instances the number of colonies obtained from 0.00001 mg. was between 40 and 200.

EXPERIMENTAL

Most of the experiments were on the protective effect of the intradermal inoculation of rabbits with heat-killed bovine (Ravenel) or human (Jamaica 22) tubercle bacilli against subsequent infection with a small dose (0.00001 mg.) of virulent bovine tubercle bacilli (Ravenel strain). A few animals were infected with a larger dose (0.01 mg.) of the same strain (Table I). The disease produced was chronic, few animals dying within less than 3 months. Animals were not killed unless *in extremis* until 2 years from the date of infection, at which time the experiments were concluded. Both human and avian tubercle bacilli produced tuberculosis in the eyes of a smaller number of rabbits.

EXPERIMENTAL OCULAR TUBERCULOSIS IN NORMAL AND IMMUNIZED RABBITS *

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The experimental work on ocular tuberculosis has been so completely reviewed by both Finnof¹ and Woods² that only studies pertaining directly to our problem will be discussed.

Kostenitsch and Wolkow³ in 1893 described the development of ocular tuberculosis in the rabbit following an intravenous injection of avian tubercle bacilli. Friedrich and Nösske⁴ in 1899, Stock⁵ in 1903, and Daels⁶ in 1907 have reported the production of ocular tuberculosis in a fairly high percentage of rabbits following intravenous injections of large numbers of tubercle bacilli. The intravenous method, however, has been replaced for the most part by intracarotid injection. Lagrange⁷ in 1898 was the first to report the localization of tubercles in the eye after the injection of tubercle bacilli into the carotid artery. The same method was used by Finnof⁸, who injected killed tubercle bacilli by this route and produced tubercles in the choroid. We have been able to confirm his results in this laboratory. Our experiments, like those of Finnof, indicate that the organisms do not localize in the eye if they are injected in the form of a fine suspension. In fact, a very coarse suspension is necessary in order to produce ocular tuberculosis by this method. Insufficient attention seems to have been given to this finding.

There are numerous reports on the effect of sensitization on the development of ocular tuberculosis. The studies of Lagrange,⁹ of Samojloff,¹⁰ and of Woods, Burky and Friedenwald¹¹ should be mentioned. These observers have described the effect of general sensitization on the intra-ocular reactions to either tubercle bacilli or tuberculin. As far as we have been able to ascertain,

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entire group, whereas it was 49.1 per cent in the nonimmunized rabbits. It is of interest that the difference in the incidence of ocular tuberculosis between the infected controls and immunized rabbits is about 2.96 times its standard deviation.

TABLE I

The Incidence of Ocular Tuberculosis in Normal and Immunized Rabbits Infected Intravenously with Bovine Tubercle Bacilli (Ravenel)

Groups	Infecting dose (mg.)	Number of rabbits	Ocular tuberculosis		Average length of life (days)
			Number	Per cent	
Infected controls.....	0.00001	57	28	49.1	188.7
Treated with heat-killed Ravenel....	0.00001	43	14	32.6	313.4
" " (4 injections)	0.00001	20	2	10.0	278.4
" " (4 injections)	0.00001	43	10	23.3	211.6
" " (1 injection)	0.00001	30	11	36.7	337.6
Treated in various ways.....	0.01	12	10	83.4	88.0
Infected controls.....	0.01	14	8	57.1	87.7
Treated with heat-killed Ravenel....	0.01				
(6 injections)					

When a larger infecting dose (.01 mg.) of tubercle bacilli was injected intravenously, the incidence of tuberculosis of the eye was greater and the average length of life considerably less. Ocular tuberculosis developed in 10 of 12 infected control rabbits and in 8 of 14 of those immunized and subsequently infected. This small group of animals will not be included in the tables or observations that follow.

Time of Development of Ocular Tuberculosis. In 18 of 28 infected control rabbits that developed ocular tuberculosis, the lesion appeared sooner than 164 days following infection, whereas it was found within this period of time in only 4 of 37 immunized rabbits (Table II). Moreover, the earliest example of ocular tuberculosis found at autopsy in the group of infected controls was at 93 days after infection, and in the immunized group at 157 days. These observations show that there is a considerable delay in the development of ocular tuberculosis, especially in the immunized group. In many instances the tubercles found in the eye were apparently recent, and it is probable that the tuberculosis in the eye was a secondary development rather than a direct

Since the ocular tuberculosis will be described in considerable detail, other findings will be briefly summarized. The chief difference between the immunized and infected control rabbits was that in the former group some animals survived infection, and the average duration of life was greater. No consistent differences were noted in the extent or character of the tuberculosis in the two groups. Usually, the most extensive involvement was in the lungs; however, lesions of other viscera, especially appendix, ileocecal junction and kidneys, were often conspicuous. Involvement of the spleen and liver was infrequent. Pulmonary cavitation was not unusual, and lesions with some calcification were often found. Histologically, the tuberculous lesions were similar to those in humans save for the relative scarcity of giant cells. There was often extensive amyloidosis which occurred chiefly in the spleen, kidneys and liver.

The eyes, removed from the animals at autopsy, were placed in formalin. They were opened by a median incision, and after removal of the vitreous humour and lens, were examined for gross evidence of tuberculosis, the size and anatomical location of any tubercles being noted. Routine histological sections were prepared from most eyes, and all lesions about which there was any doubt were studied microscopically. When these examinations were completed, the ocular tuberculosis was studied in relation to the time of development, duration of life, amount of pulmonary tuberculosis, degree of sensitization and the presence of complement-fixing antibodies.

RESULTS OF EXPERIMENTS

The number of rabbits, the method of immunization, the amount of the infecting dose, the average duration of life and the number of animals developing ocular tuberculosis are shown in Table I.

From this table it is evident that the immunized animals in the groups receiving an infecting dose of 0.00001 mg. lived, on the average, longer than did the infected controls. The incidence of tuberculosis of the eye was also considerably less. In the four groups of immunized animals the incidence of ocular tuberculosis varied from 10 to 36.7 per cent, and was 27.2 per cent for this

control and immunized rabbits are considered together, ocular tuberculosis is found in 29 of 116, or 25.0 per cent, of those with less than half of the lung tissue involved, and in 36 of 71, or 50.7 per cent with more than half involved. In other words, the ocular tuberculosis occurred in direct proportion to the amount of tuberculosis in the lungs. However, in some instances it developed in the eyes when there was relatively scant pulmonary tuberculosis.

Relationship to Sensitization. Tuberculosis of the eye was next considered in relation to the amount of skin sensitivity to tuberculin. In the infected control group we have considered the highest tuberculin reading subsequent to infection as the index of sensitivity for each animal although this may have changed considerably just prior to death. For the immunized group, the tuberculin test just prior to infection has been used. On this basis the animals with ocular tuberculosis and those without were considered in relation to the degree of tuberculin skin sensitiveness. So far as we were able to determine, the degree of skin sensitivity did not bear any relationship to the occurrence of ocular tuberculosis.

Complement Fixation. Complement fixation tests were done on all the animals, but no relationship was found between antibody formation and the development of ocular tuberculosis.

PATHOLOGICAL LESIONS

In a few instances tubercles were observed in the iris and anterior chamber of the eyes during life (Figs. 1-5). The location and size of the tubercles were noted at autopsy and the location is shown diagrammatically in Text-Figure 1. Tubercles were found to occur most frequently in the posterior part of the iris and in the region of the ciliary body. When the eyes were extensively involved, the tubercles in the ciliary body were frequently large and caseous and perforated the eye at the corneo-scleral junction. The tubercles in the choroid were readily seen through the retina and measured from pin point size up to about 7 mm. in diameter. They occurred in the ciliary processes frequently enough to suggest that these processes may be of significance in causing bacteria to localize in the eye. The significance of this relation has been pointed out in an earlier publication.¹³

result of the original intravenous injection. Some studies made by Deane C. Hartman in this laboratory are of interest in this connection and tend to support this observation. He made repeated ophthalmoscopic examinations of the eyes of an additional large group of infected rabbits for a period of more than 1 year following infection. In no instance did he observe tuberculosis

TABLE II
*The Time of Development of Ocular Tuberculosis
in Infected Controls and Immunized Rabbits*

Groups	No. of rabbits	No. developing ocular tuberculosis	
		Within 164 days	After 164 days
Infected controls.....	28	18	10
Immunized rabbits.....	37	4	33

in the eyes of these rabbits sooner than 5 months after infection. If it were the result of the original injection, it would probably have developed before this time.

Relationship to Pulmonary Tuberculosis. At the time of autopsy the amount of tuberculosis in the lungs of all the rabbits was recorded in percentage of the cut surface of the lungs occupied by tuberculous lesions. It seemed desirable to determine whether the ocular tuberculosis bore any relationship to the amount of pulmonary tuberculosis. The animals were accordingly separated into two groups, depending upon the extent of

TABLE III
*Incidence of Tuberculosis of the Eyes of Rabbits
in Relation to the Extent of Lung Involvement*

Groups	Per cent of lung involvement	No. of rabbits	With ocular tuberculosis	
			Number	Per cent
Infected controls.....	50% or less	36	13	36.1
	More than 50%	21	15	71.4
Immunized animals.....	50% or less	80	16	20.0
	More than 50%	50	21	42.0

lung involvement. The results are shown in Table III. It is evident that the incidence of ocular tuberculosis in both the infected controls and immunized rabbits is considerably smaller in animals in which less than 50 per cent of the lung is involved than in rabbits in which more than 50 per cent is involved. When the

in those of the choroid. Giant cells were relatively rare and were observed in only three of the eyes sectioned.

Sections stained by the Ziehl-Neelsen method were prepared in many instances and large numbers of acid-fast bacilli were found in the tubercles in all the eyes examined in this manner. Examples of the various types of tuberculosis observed are illustrated by photomicrographs (Figs. 6-11).

SUMMARY AND CONCLUSIONS

1. Tuberculosis developed in the eyes of 28 (49.1 per cent) of 57 infected control rabbits injected intravenously with 0.00001 mg. of bovine tubercle bacilli.

2. Tuberculosis developed in the eyes of 37 (27.2 per cent) of 136 rabbits immunized in various ways with either heat-killed human or bovine tubercle bacilli and subsequently infected with 0.00001 mg. of bovine tubercle bacilli.

3. Larger amounts (0.01 mg.) of bovine tubercle bacilli produced ocular tuberculosis in 10 (83.4 per cent) of 12 infected controls as contrasted with 8 (57.1 per cent) of 14 immunized rabbits.

4. Tuberculosis was not observed in the eyes of rabbits infected with 0.00001 mg. of bovine tubercle bacilli sooner than 3 months after infection. It appeared considerably earlier in the infected controls than in the immunized animals.

5. The incidence of ocular tuberculosis was closely related to the amount of tuberculosis in the lungs.

6. Tubercles were observed most often in the anterior segment of the eyes and occurred relatively frequently in both the ciliary body and ciliary processes.

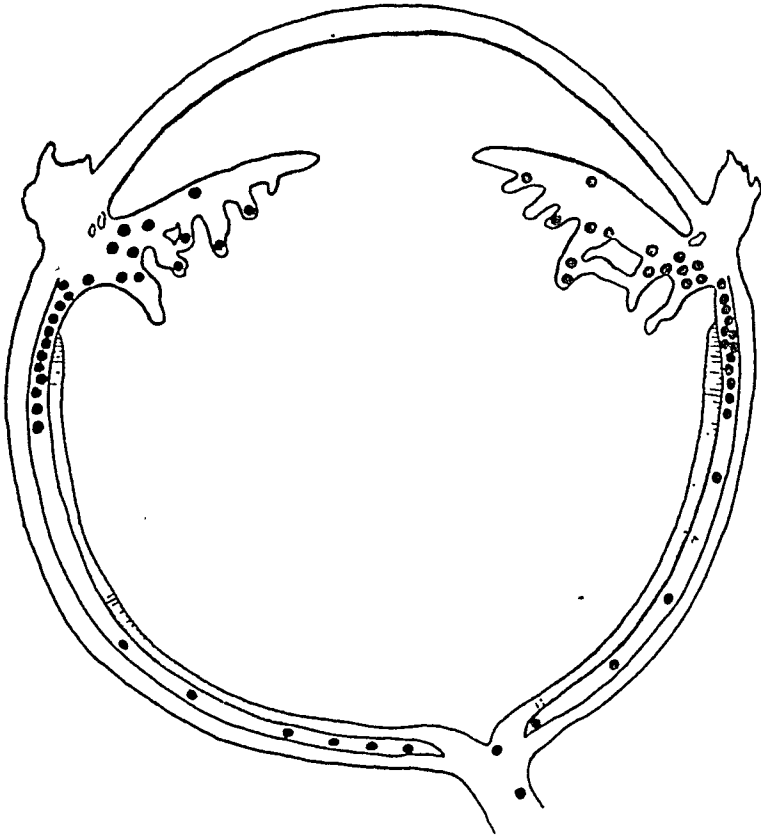
7. Skin sensitivity to tuberculin did not seem to have any influence on either the gross or microscopic characteristics of tuberculosis in the eyes.

8. These experiments indicate that tuberculosis develops frequently in the eyes of infected rabbits. Immunization preceding infection affords some protection against the occurrence of ocular tuberculosis.

NOTE: We wish to thank Jules Freund and R. W. Linton for their help in accumulating this material.

Tubercles in smaller numbers were present in the anterior part of the choroid and in two instances involved the optic nerve.

The microscopic sections of eyes with tuberculosis from the infected controls were compared with those from the immunized rabbits with special reference to the size of the tubercles, type of cellular reaction, amount of fibrous connective tissue, degree



Text-Figure 1. Distribution of ocular tubercles as found at autopsy.

of caseation, number of giant cells and increase in pigmented cells. No significant difference was noted in the type of inflammatory reaction. The microscopic findings apparently depended largely upon the age and size of the tubercles. In a considerable number there were many polymorphonuclear leukocytes. Epithelioid cells and lymphocytes were present in practically every instance. There was a considerable amount of fibrous connective tissue in the tubercles of about one third of the eyes. There were varying degrees of caseation. Frequently, large numbers of pigmented chromatophores were present in the tubercles, especially

DESCRIPTION OF PLATES

PLATE 32

FIGS. 1 and 2. Rabbit No. 1. Tuberculosis of the iris. Both eyes have been fixed in 10 per cent formalin and the corneas removed. This rabbit had been immunized with four intradermal injections of 0.025 mg. of heat-killed bovine tubercle bacilli (Ravenel) and infected with 0.00001 mg. of bovine tubercle bacilli. The rabbit died 383 days after it was infected.

FIG. 3. Rabbit No. 2. A tubercle in the anterior chamber near the iris. This rabbit was treated in the same manner as rabbit No. 1. The photograph was taken approximately 22 months after infection. The highlights have been removed.

FIGS. 4 and 5. Rabbit No. 3. Extensive tuberculosis of both eyes in an infected control that received 0.01 mg. of human (Jamaica 22) tubercle bacilli intravenously. Photograph was taken about one year later. *Right eye*: There is extensive clouding of the cornea, with extensive vascularization at the corneoscleral junction, most marked on the lateral aspect. *Left eye*: There is considerable clouding of the cornea, as well as several large tubercles in the anterior chamber. A highlight in the photograph has been obliterated to avoid confusion with the tubercles.

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PLATE 33

FIG. 6. Rabbit No. 4. A large tubercle involving most of the ciliary body and part of the iris. This rabbit died from tuberculosis 164 days after an intravenous injection of 0.00001 mg. of bovine tubercle bacilli. $\times 13$.

FIG. 7. Rabbit No. 5. Tubercles in the ciliary processes on the posterior part of the iris. This rabbit died from tuberculosis 160 days after receiving an intravenous injection of 0.00001 mg. of bovine tubercle bacilli. $\times 100$.



FIG. 1

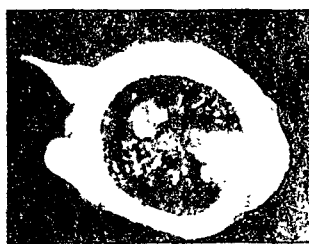


FIG. 2



FIG. 3



FIG. 4

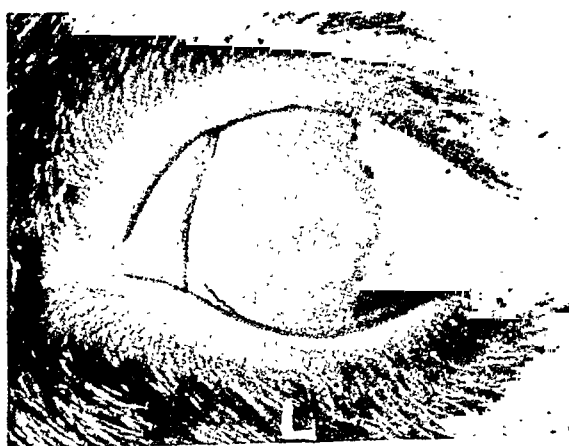


FIG. 5

Experimental Ocular Tuberculosis

Angevine and Huntington

PLATE 34

FIG. 8. Rabbit No. 6. A tubercle in the iris composed largely of epithelioid cells, lymphocytes and pigmented cells. This rabbit died from tuberculosis 180 days following an intravenous injection of 0.00001 mg. of bovine tubercle bacilli. $\times 100$.

FIG. 9. Rabbit No. 7. Several small localized tubercles in the iris composed largely of mononuclear leukocytes. This rabbit had been immunized with one injection of killed human tubercle bacilli and subsequently infected with an intravenous injection of 0.00001 mg. bovine tubercle bacilli. The animal died 230 days following infection. $\times 180$.



6

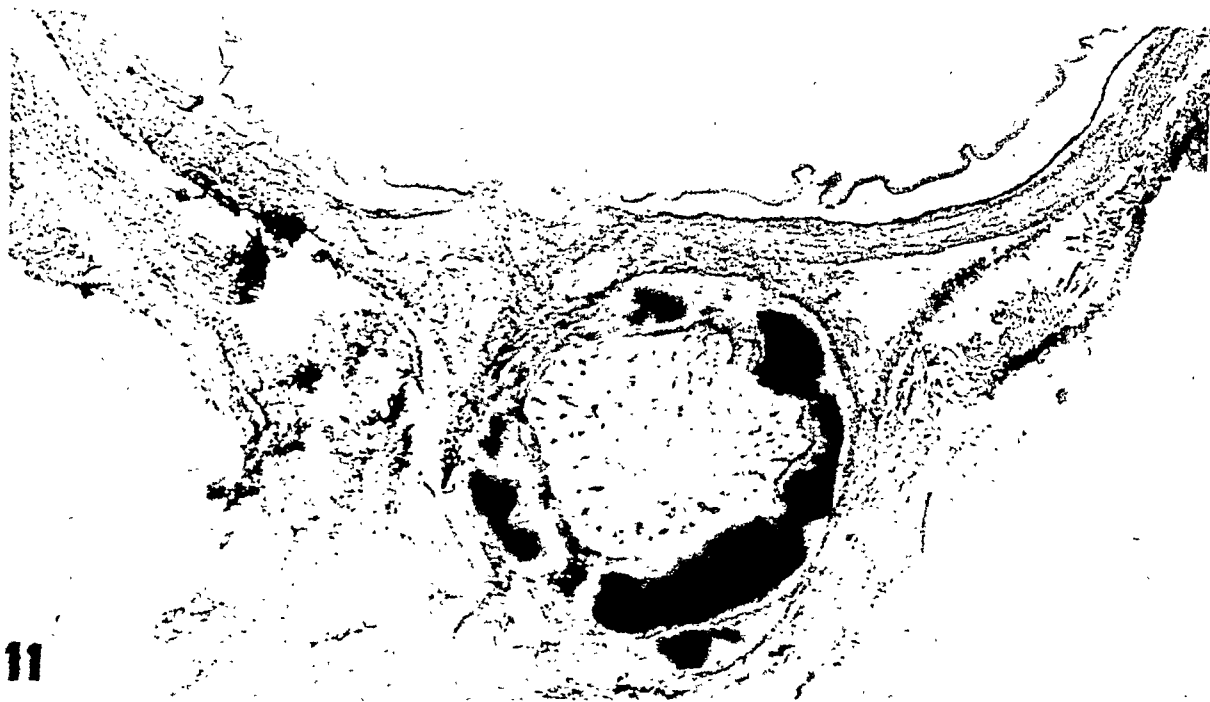
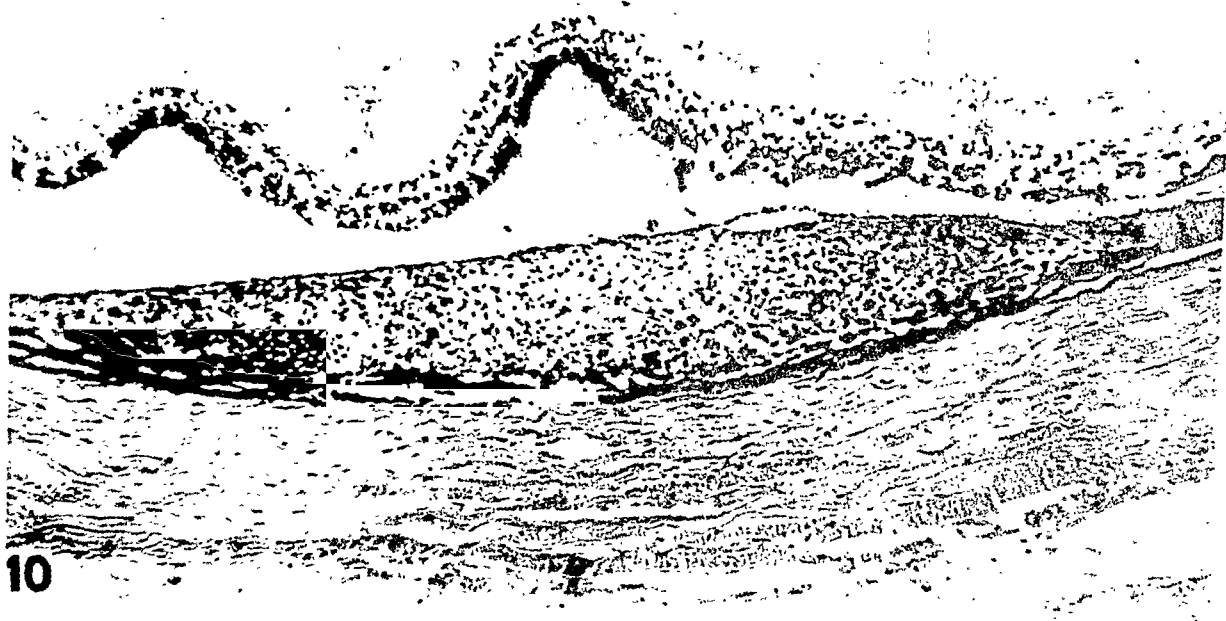


7

PLATE 35

- FIG. 10. Rabbit No. 8. A tubercle in the choroid. Note the large number of pigment-containing cells and also evidence of edema beneath the retina. This rabbit died from tuberculosis 156 days after an intravenous injection of 0.00001 mg. of bovine tubercle bacilli. $\times 90$.
- FIG. 11. Rabbit No. 9. Several large tubercles with beginning caseation surrounding the optic nerve. The nerve tissue is extensively infiltrated with lymphocytes. The rabbit was treated in the same manner as rabbit No. 7 and died 214 days after infection. $\times 15$.





the more likely in view of the fact that some of these authors reported transmission of measles to mammals and birds of several species generally regarded as resistant.

Reports as to the transmissibility of measles from man to guinea pigs are equally conflicting. Duval and D'Aunoy³ noted leukopenia, fever, hemorrhagic nephritis and inconstant coryza in their animals. They found that the virulence of the virus was increased by serial animal passage. Some of the animals inoculated with the more potent virus died, while others recovered and were immune to subsequent inoculation. Enders¹⁰ stated that Ueda and Kasahara¹¹ also produced systemic changes in guinea pigs injected with blood removed from patients with measles. They found an interstitial pneumonia, with perivascular infiltrations of large "mononuclear cells," in their animals and produced clinical signs of measles in monkeys injected with emulsions of the lungs and testes of infected guinea pigs. Enders was unable to confirm these results and found similar pneumonic lesions in control guinea pigs. Negative results with guinea pigs have been reported also by Kraft,⁹ Nicolle and Conseil¹² and Blake and Trask.¹³

It is more generally agreed that monkeys are susceptible, at least in a mild form. Blake and Trask¹³ and Blake, Trask, Culotta and Beebe¹⁴ injected monkeys intratracheally with nasopharyngeal washings obtained from patients with measles. Their animals, after a short period of incubation, developed an enanthem, malaise, conjunctivitis and eruption. The lesions found in the skin and mucous membranes showed histologic changes similar to those described by Mallory and Medlar¹⁵ in Koplik's spots and in the cutaneous lesions of measles in man. The virus could be maintained for a limited period by animal passage. Successful inoculation of monkeys has been reported by others.^{1,6,10,16} Rake and Shaffer¹⁶ propagated the virus of measles on the chorio-allantois of chick embryos and infected monkeys with the virus grown in this manner. They were able to recover the virus from monkeys thus infected and propagate it again by incubation in fertilized eggs.

A few investigators^{17,18,19} have found monkeys resistant to inoculation with blood or nasal secretions obtained from patients with measles. Sellards and Wentworth¹⁸ injected five monkeys

EXPERIMENTAL MEASLES *

THE LYMPHOID TISSUES OF ANIMALS INOCULATED WITH THE VIRUS OF HUMAN MEASLES

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The prodromal stage of measles in man is accompanied by a specific giant cell reaction in lymphoid tissues. The present study was planned to utilize this reaction as a criterion of the transmissibility of measles from man to animals.

LITERATURE

Numerous attempts have been made to produce measles in animals. Conflicting results were obtained when rabbits and guinea pigs were used as subjects and clinical and hematologic data were made the criteria of success or failure. Successful transmission of measles from man to rabbits, based on clinical manifestations, has been claimed by several investigators.¹⁻⁴

Nevin and Bittman¹ injected rabbits intramuscularly with blood obtained from patients with measles in the early eruptive stage. The rabbits developed signs of measles lasting from 5 to 14 days. Control rabbits receiving blood from healthy donors or from patients with diseases other than measles did not develop similar clinical signs. Nevin and Bittman passed the virus serially through five rabbits and produced clinical signs of measles in a monkey injected with blood drawn from the fifth rabbit.

Several workers,⁵⁻¹⁰ on the other hand, have found rabbits immune to measles. Purdy⁶ showed that leukopenia and temperature variations similar to those considered significant by Scott and Simon,⁴ may occur in normal rabbits. Enders¹⁰ suggested that the "positive" results reported by a number of Japanese investigators may have been due to accidental infection by a virus other than that of measles. This explanation appears

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the subepithelial layer and in the germinal centers." He looked upon these cells as a defensive mechanism and considered it likely that they contain the infectious agent of measles. Finkeldey²³ suggested that the reaction may be in the nature of an allergic response. Cells of a similar nature and significance have been found in the appendix.²⁸⁻³² Hathaway³⁴ found them in the spleen and lymph nodes of a child who died of peritonitis during the prodromal stage of measles. Multinucleate giant cells containing cytoplasmic inclusion bodies have been found in the respiratory tract during the prodromal stage of measles³⁵ and Masugi and Minami³⁶ reported the presence of giant cells in the epithelium of the respiratory tract 4 days after the development of the rash of measles. Stryker³⁷ recently questioned the specificity of the giant cells described by Masugi and Minami. He found four distinct types of giant cells in the lungs and respiratory tract, but considered only one of these specific for measles.

With some of these reports in mind, it was decided to inoculate a number of animals with blood taken from patients with measles and examine their organs for the presence of Warthin-Finkeldey giant cells.

EXPERIMENTAL

Material and Methods. The following experiments were performed at intervals from 1938 to 1940, whenever the necessary clinical material became available. In all, 5 patients were used. These were negative, both clinically and serologically, for syphilis. All had unmistakable signs of measles, the first 3 in a severe, the last 2 in a mild form. The diagnosis, in each instance, was substantiated by an experienced clinician. Patients Nos. 1, 2 and 3 were children; Nos. 4 and 5 young adults. Blood was taken from them within 24 hours after the appearance of the rash and "citrated." A portion was cultured aerobically and anaerobically on blood agar and in Douglas broth. There was no growth in any of the cultures. The remainder of the blood was injected immediately into rabbits, white rats, guinea pigs and monkeys, as follows: On April 12, 1938, 4 rabbits were injected intravenously with blood obtained from patient No. 1 and on April 15, 4 with blood removed from patient No. 2. One rabbit in the second group received 4 cc., the other 7 rabbits 2.5 cc. each. On April

intraperitoneally with blood. Two of the monkeys developed leukopenia and one of these a slight rash. Blood taken from the latter at the onset of the leukopenia and 5 days before the appearance of the rash failed to cause measles when injected into an adult human volunteer. This is in contrast to the results obtained by Nicolle and Conseil¹² who reported the production of measles in a child injected with blood taken from a monkey which had been infected with measles experimentally. Degkwitz²⁰ and Degkwitz and Mayer²¹ produced definite clinical signs of measles in only 10 per cent of their monkeys and Kraft⁹ had equally disappointing results.

In summary it may be said that a majority of observers agree that monkeys are susceptible to measles. Opinion as to the susceptibility of animals below the order of primates is more sharply divided. A majority of the authors cited have found guinea pigs and rabbits to be immune.

Much of the uncertainty indicated in these reports could have been dispelled had search been made for the histologic lesions described independently by Warthin²² and by Finkeldey²³ in 1931. This omission is hardly surprising, however, in view of the fact that a description of these lesions is omitted in recent editions of three widely used textbooks of pathology,^{24,25,26} while a fourth²⁷ mentions them only briefly. The diagnostic significance of the Warthin-Finkeldey giant cell reaction has been confirmed on many occasions.²⁸⁻³² Its presence may be accepted as a sign of impending measles and it probably does not occur in any other infection. This statement is made despite the recent report of Tomlinson³³ who found similar cells in tonsils removed from a child who subsequently developed chickenpox. It is probable that this patient had measles in such a mild form as to be unrecognized, the chickenpox developing as an intercurrent infection.

The Warthin-Finkeldey giant cell is a large, multinucleate, round or irregular syncytial cell. It has a variable number of lightly staining nuclei characteristically arranged in a grapelike cluster near the center of the cell.²² In the tonsil these cells are most numerous beneath the epithelium of the crypts and that of the surface, but also occur within the germinal centers of the lymphoid follicles. Warthin suggested that they arise by "amitotic division in hyperchromatic cells resembling lymphocytes in

ning temperatures for a period of 14 days after injection. Total and differential counts of the white blood cells were made.

From each of the 4 monkeys, an inguinal lymph node was removed immediately before injection and 5 additional nodes at intervals thereafter. Thus a lymph node was removed from monkey No. 1 on the fourth, sixth, eighth, tenth and eleventh days; from No. 2 on the third, fifth, seventh, ninth and eleventh days; from No. 3 on the third, fifth, seventh, tenth and twelfth days; and from No. 4 on the fourth, sixth, eighth, eleventh and fourteenth days after inoculation (Table I). The lymph nodes were fixed in 4 per cent aqueous solution of formaldehyde, embedded in paraffin and stained with hematoxylin and eosin. Several sections were cut from each node and examined microscopically. The monkeys were kept under close clinical observation for 1 month after inoculation, search being made daily for signs of enanthem, exanthem, conjunctivitis or coryza.

TABLE I

The Occurrence of the Giant Cell Reaction in the Lymph Nodes of Monkeys which Had Received Human Blood

Monkey No.	Date inoculated	Source of blood	Amount injected	Giant cell reaction in lymph nodes Days after inoculation											
				Control	3	4	5	6	7	8	9	10	11	12	14
1	5-12-39	Pt. No. 4	6 cc.	—		+		+		+		±	—		
2	5-12-39	Pt. No. 4	6 cc.	—	+		+		+		+		—		
3	5-3-40	Pt. No. 5	10 cc.	—	—		—		+			+		—	
4	5-3-40	Pt. No. 5	10 cc.	—		—		—		—			—		—

Results. None of the rats, guinea pigs or rabbits exhibited clinical signs indicative of measles and there were no significant changes in the temperature of the rabbits. The blood counts of the rabbits fluctuated within wide limits, but similar fluctuations occur in healthy rabbits.³⁸ None of the lymph nodes removed from the rabbits contained giant cells of the Warthin-Finkeldey type, nor were any seen in the tissues removed at autopsy from the rats, guinea pigs or rabbits. None of the monkeys developed coryza, conjunctivitis or exanthem during the 4-week period of observation. Monkey No. 3, which received 10 cc. of blood from patient No. 5, developed a small, whitish papule on the labial mucous membrane opposite the left lower central incisor on the sixth day after injection. This became hyperemic the next day

18, blood was taken from patient No. 3 and injected into 1 rabbit, 5 white rats and 3 guinea pigs. The rabbit received 6 cc. intravenously, the white rats 2 cc. each, intracardially. Two of the guinea pigs were injected by the intracardiac, the other by the intraperitoneal route, each receiving 1.5 cc. From patient No. 4, 6 cc. of blood was injected into each of 2 monkeys on May 12, 1939. Blood from patient No. 5 was injected in 10 cc. portions into each of 2 monkeys on May 3, 1940. The injections were made intravenously.

Two popliteal lymph nodes were removed from each of 6 of the rabbits. These were taken from the third to the eighth day, inclusive, after inoculation, so that continuous daily biopsies were available during that period. Sixteen of the 20 rabbits used in these experiments, and in that described below, were sacrificed for necropsy at intervals of 6, 7, 8, 9 and 11 days, respectively; 3 others were sacrificed 1 month after inoculation. The rats were sacrificed 4, 6, 8 and 11 days, and the guinea pigs 6 and 11 days, after injection. Sections were taken from the lymph nodes, parenchymatous organs, respiratory and gastro-intestinal tracts of all animals. All tissues, whether derived from biopsy or necropsy, were fixed promptly in 4 per cent aqueous solution of formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

An additional experiment, designed to repeat the work of Duval and D'Aunoy³ who claimed to have increased the virulence of the virus of measles by animal passage, was performed as follows: On April 18, 1938, that is, 3 and 6 days after their inoculation, 2 of the rabbits which had been injected with the blood of patient No. 1, and 2 with blood from patient No. 2, were bled. The blood was "citratized," pooled and injected intravenously into 3 healthy rabbits, each of which received 6 cc. Seven days later blood was removed from each of these rabbits and injected into 3 fresh rabbits. A week later blood was taken from these and injected into 3 additional rabbits. Thus the agent received by the third group had been passed serially through three groups of rabbits. Two other rabbits were injected with blood from normal rabbits as a control. The animals used in this, as in all the other experiments, were kept under close clinical observation and a daily record made of their morning and eve-

considered pathognomonic of impending measles in man. It was absent from the control nodes removed immediately before inoculation. It appeared after an incubation period and disappeared after an interval compatible with clinical experience with measles in man. Above all, the fact that no giant cells were seen in the lymph nodes removed from monkey No. 4, indicates that the reaction is not due to the injection of blood *per se*. It is interesting that none of the monkeys, with the possible exception of No. 3, exhibited clinical signs of measles. This may be accounted for in one of two ways: either the virus used by us was attenuated or the monkeys were relatively resistant. In either case it is not unlikely that the Warthin-Finkeldey giant cell reaction is a much more sensitive indication of infection than are clinical manifestations. In man, infection may be so mild that a diagnosis of measles cannot be made with certainty. This was true of one of the cases reported by Warthin,²² and may be the explanation for the changes reported by Tomlinson.³³ It is planned to test two of the monkeys later for immunity. It is also planned to study tissues from chick embryos inoculated with blood from patients with measles. The present series of experiments is reported now in order to stimulate similar studies by others.

An additional point is worthy of emphasis. Occasionally the plane of a section passes tangentially through the wall of a small vessel. In such cases the nuclei of the endothelial cells appear clumped, producing a formation suggesting a multinucleate giant cell. It is rather easy to distinguish between the Warthin-Finkeldey and the fictitious giant cell. The nuclei of the latter are arranged in a much more orderly manner than those of the former and the lumen of the vessel becomes apparent in consecutive sections. The Warthin-Finkeldey giant cell, on the other hand, always shows its typical cluster of nuclei and never a lumen.

SUMMARY

Blood taken from patients with measles was injected into white rats, guinea pigs, rabbits and monkeys.

The animals were kept under clinical observation and their tissues examined for Warthin-Finkeldey giant cells.

Histologically, there was no evidence that measles can be transmitted from man to white rats, rabbits or guinea pigs.

and disappeared 3 days later. It never showed the bluish central zone of a typical Koplik's spot, but was nevertheless somewhat suggestive of an enanthematous lesion. The animal was listless and had slight chills from the ninth to the eleventh day, inclusive, after inoculation. No lesion of an enanthematous nature was seen in any of the other monkeys.

Sections of the lymph nodes, removed from the monkeys before injection, showed no microscopic pathology other than slight localized hemosiderosis in monkey No. 2. Those removed after inoculation all revealed some degree of hyperplastic lymphadenitis. In addition, Warthin-Finkeldey giant cells were present near areas of reticulum hyperplasia and occasionally in the follicles of some of the lymph nodes taken from monkeys Nos. 1, 2 and 3. These giant cells varied considerably in appearance. Some, apparently recently formed, were small and appeared to be derived from monocytes which had multiplied by amitotic division (Figs. 1 and 2). Others were of such size as to be present in several consecutive sections cut approximately $6\ \mu$ thick. These had a variable number of pale, vesicular nuclei arranged in a cluster near the center of the cell, of which the cytoplasm was syncytial and faintly granular in character (Figs. 3 and 4). Some lymph nodes, removed from 2 to 6 days after the initial appearance of the Warthin-Finkeldey reaction, contained giant cells with hyaline cytoplasm and pyknotic nuclei (Figs. 5 and 6). These cells resembled megakaryocytes. These changes are interpreted as indicative of involution. None of the lymph nodes contained as many Warthin-Finkeldey giant cells as are commonly seen in human tissues. Occasionally, two were seen in close proximity (Figs. 3 and 4) and sometimes three (Fig. 7). The reaction lasts from 3 to 6 days, appearing as early as the third or as late as the seventh day after inoculation.

DISCUSSION

On the basis of the clinical and histological data presented, white rats, guinea pigs and rabbits are not susceptible to the virus of human measles. Some monkeys, on the other hand, do appear to be susceptible.

The giant cell reaction described and illustrated in the lymph nodes removed from the monkeys, is almost identical with that

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Blood originally drawn from patients with measles was passed serially through three rabbits and injected into normal rabbits. The latter showed no clinical or histological evidence of measles.

One monkey out of four injected with human blood developed a questionable enanthem 6 days after inoculation. This lesion disappeared spontaneously 3 days later. This animal appeared listless and had chills from the ninth to the eleventh day after inoculation. The other three monkeys exhibited no clinical signs of measles.

Giant cells were found in the lymph nodes of three of four monkeys injected with blood removed from two patients with mild attacks of measles. These giant cells resemble those described by Warthin and by Finkeldey in tonsils removed during the prodromal stage of measles.

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DESCRIPTION OF PLATES

PLATE 36

- FIG. 1. Lymph node removed from monkey No. 2, 5 days after injection with 6 cc. of blood taken from patient No. 4. Near the center are two giant cells. There is a diffuse proliferation of monocytes and reticulum cells in the pulp. Hematoxylin and eosin stain. $\times 130$.
- FIG. 2. Higher magnification of a portion of the area included in Figure 1, to show the character of the giant cells and their probable origin from monocytes. $\times 440$.
- FIGS. 3 and 4. Lymph node removed from monkey No. 2, 9 days after injection. These photographs are made from consecutive sections, cut about $6\ \mu$ thick. There is a distinct difference in the configuration of the giant cells in the two photographs. The clumping of the nuclei and the syncytial character of the cells is very evident. Hematoxylin and eosin stain. $\times 460$.

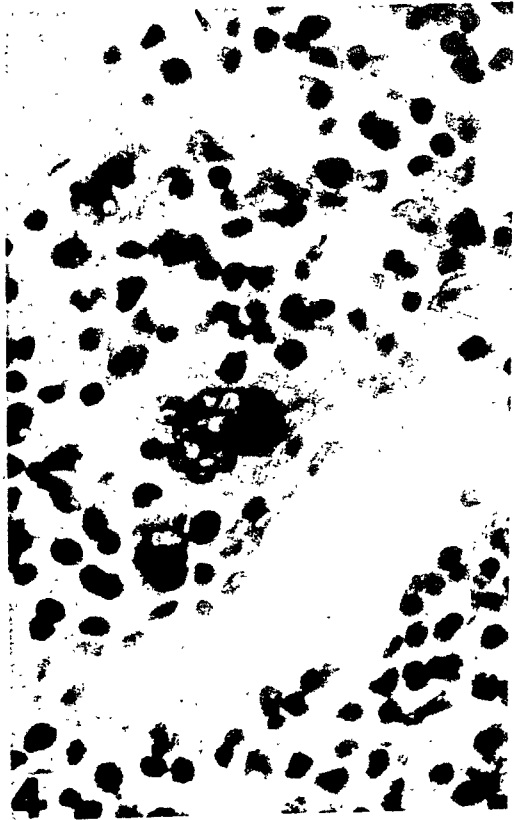
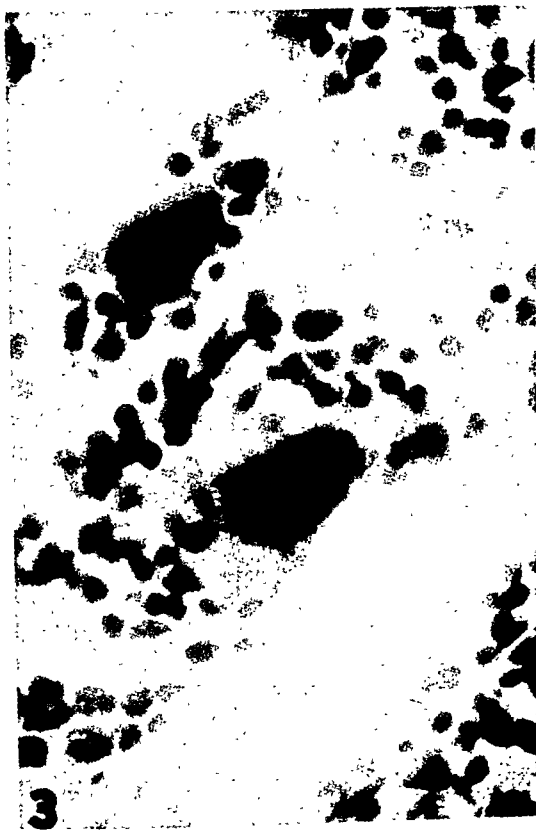
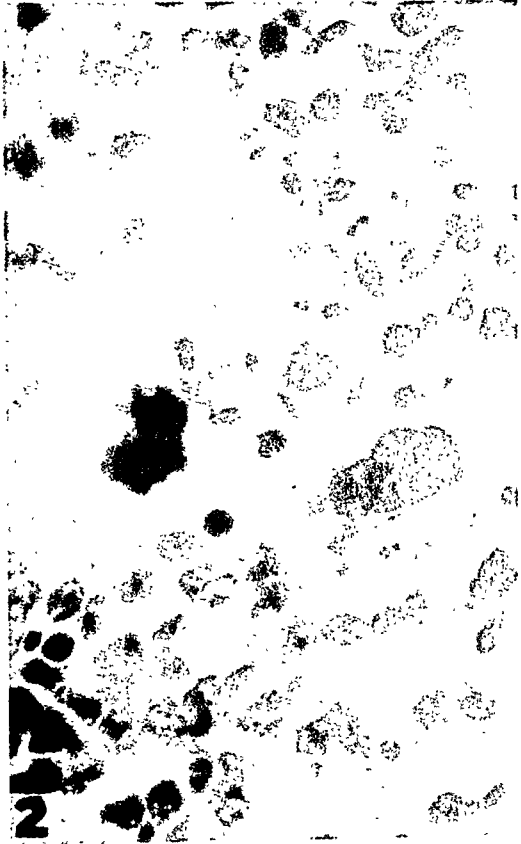
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PLATE 37

FIG. 5. From a lymph node removed from monkey No. 3, 10 days after injection with 10 cc. of blood taken from patient No. 5. The cytoplasm of one giant cell is hyaline and its nuclei are pyknotic. Hematoxylin and eosin stain. $\times 130$.

FIG. 6. From a lymph node removed from monkey No. 1, 10 days after injection with 6 cc. of blood taken from patient No. 4. Below the center is an involuting giant cell with little cytoplasm and pyknotic nuclei. Hematoxylin and eosin stain. $\times 130$.

FIG. 7. From a lymph node removed from monkey No. 3, 7 days after injection. Three giant cells are present in one field. The syncytial character of the giant cell near the center is apparent. Hematoxylin and eosin stain. $\times 130$.



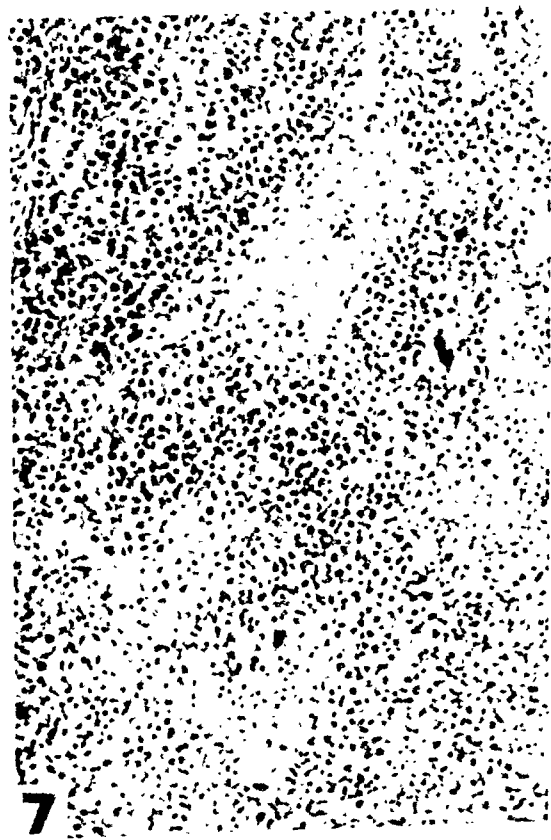
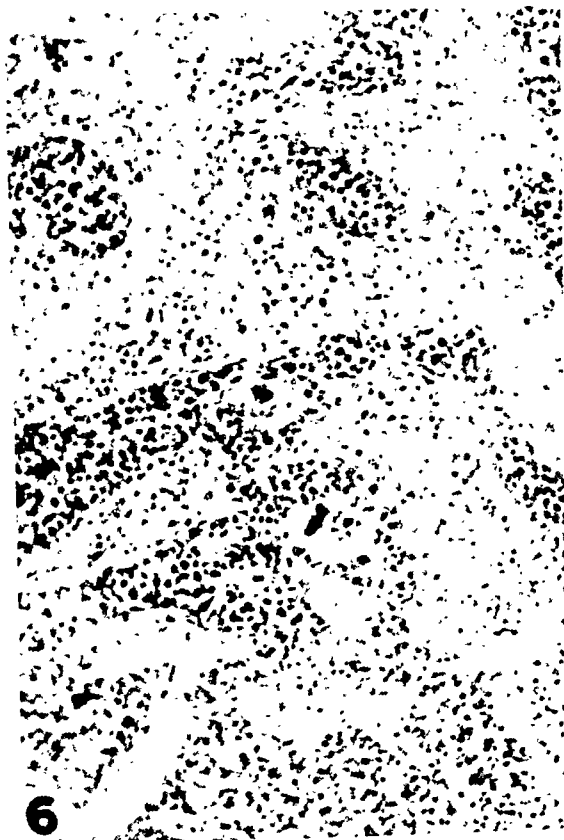
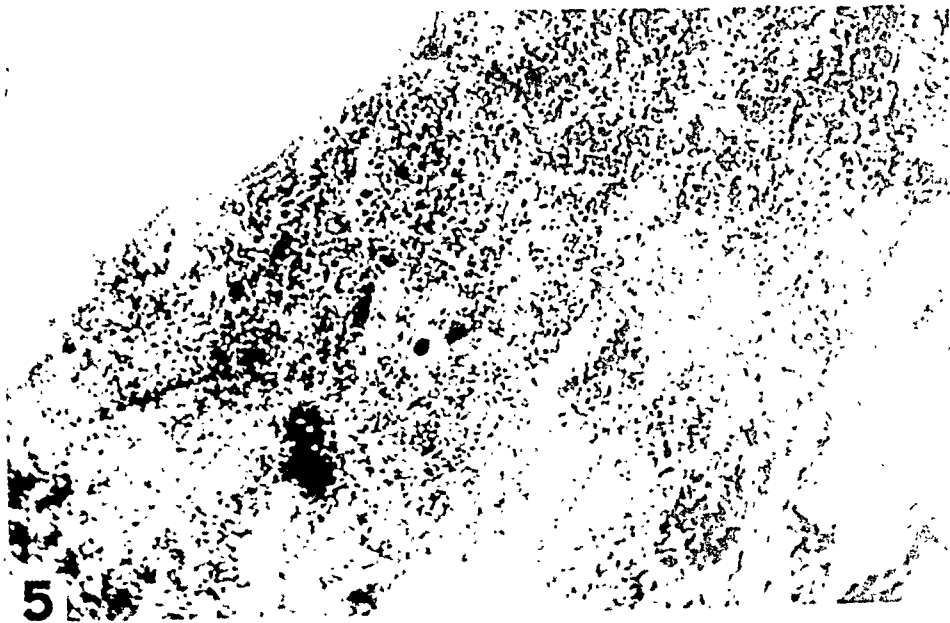


TABLE I
Histories of Three Strains of Chick-Embryo-Adapted Rabies Virus

	Strain C	Strain R	Strain H
Type of virus.....	street	fixed	human
Source of virus.....	dog brain	rabbit brain	human brain
Experimental hosts used in preliminary passages.....	mice day-old chicks day-old chicks day-old chicks day-old chicks	none	mice
Number of passages in embryos.....	65	25	3

13 days' incubation, are candled to locate the head and a square window 2 cm. in width is then cut in the shell as described by Goodpasture and Buddingh.⁶ A $\frac{1}{2}$ inch 27 gauge needle fitted to a 0.25 cc. syringe is used to inoculate the embryos. If the head is not accessible, the needle may be used to manipulate it but rough handling is to be avoided. Injection can be made anywhere in the brain, but in our experience the tectum is more easily inoculated. Once the head is exposed and the desired site located, a sudden short thrust will usually force the needle through the calvarium. After inoculation the window is ringed with a vaseline-paraffin mixture and closed with a coverglass.

Four days after inoculation one of the first generation embryos was killed and its brain passed to embryos 13 days old. Subsequent passages were made at intervals of 6 days. All inoculations were made in the manner described except that in later generations the volume of the inoculum was reduced to 0.03 cc. This strain, which is designated strain "C," has been carried through 65 generations in the embryo.

A second strain, established by direct inoculation of 10 per cent emulsion of rabbit-brain-fixed virus into the brains of embryos, has been maintained in this host for 25 generations. This strain is designated strain "R." The third strain was derived from a human case of rabies. An emulsion of human brain was inoculated intracerebrally in mice and pooled brains of prostrate mice served as the original inoculum for embryos. In establishing this strain it was necessary to use embryos 10 days old and to allow an incubation period of 10 days. This strain, which is designated

A STUDY OF CHICK-EMBRYO-ADAPTED RABIES VIRUS*

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Prior to the first publication from this laboratory in 1939,¹ all attempts to establish rabies in chick embryos had been unsuccessful. Others, ^{2,3,4} as well as myself, had tried to induce rabies in the embryo but in all of these experiments inoculations had been made on the chorio-allantoic membrane. The advent of new technics for direct inoculation of the embryo ⁵ has made it possible to reinvestigate the chick embryo as an experimental host for rabies virus.

MATERIALS AND METHODS EMPLOYED IN ESTABLISHING RABIES IN CHICK EMBRYOS

The histories of three strains of rabies virus which were established in embryos are summarized in Table I.

The first strain used in this study was obtained from the brain of a dog dying of street rabies. An emulsion of the animal's brain was injected intracerebrally into Swiss mice, which developed weakness and paralysis on the seventh day and prostration on the eighth day. Some were killed on the eighth day and 0.05 cc. amounts of 10 per cent emulsion of the pooled brains were injected intracerebrally into day-old chicks. The chicks developed weakness and paralysis after 17 or 18 days; some were killed, and pooled brain emulsion was passed intracerebrally to other day-old chicks. Four passages in newly hatched chicks failed to cause any significant shortening of the incubation period or any change in the clinical course of the disease.

Pooled brains from the fourth generation in chicks were emulsified to 10 per cent strength and 0.05 cc. amounts were injected into the brains of embryos 13 days old. The technic for intracerebral inoculation of embryos is as follows: fertile eggs, after

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myelomalacia of a severe degree. Inflammatory cells in the central nervous system were few, consisting chiefly of large mononuclear phagocytes, but outside the central nervous system in the dorsal root ganglia, sympathetic ganglia and in the intrinsic ganglia of the various viscera, large collections of lymphocytes were seen.

The more specific microscopical lesions consisted of Negri bodies. These inclusions, stained by the Goodpasture method,⁷ appeared as acidophilic, refractile, hyaline bodies, which varied greatly in size and in shape. In the larger bodies, vacuoles and basophilic inner bodies were demonstrable but the smaller ones were usually homogeneous. Characteristically, the inclusions were within the cytoplasm but many appeared to lie outside of the cells. These no doubt were Negri bodies situated far out in the processes of neurons or were released from the cytoplasm of disintegrated cells. These cytoplasmic inclusions were present throughout the central nervous system, in the retina and in various peripheral ganglia. They were present as early as 3 days and as late as 13 days after inoculation but they were most numerous on the sixth and seventh days. Neurons were affected chiefly but Negri bodies were also observed in ependymal epithelium. They were occasionally seen within the cytoplasm of large mononuclear phagocytes, presumably as a result of phagocytosis. No significant changes were found in the non-nervous tissue.

Negri bodies in the chick embryo are unusually numerous; most cells are involved and many cells contain several. This is especially true in the central nervous system and to a lesser degree for cells in the retina and in peripheral ganglia. The Negri bodies in the chick embryo are so numerous and so widely distributed that the diagnosis of rabies may be made from sections of any portion of the nervous system containing nerve cells.

SUSCEPTIBILITY OF CHICK EMBRYOS TO RABIES VIRUS INOCULATED BY ROUTES OTHER THAN THE INTRACEREBRAL

All routine serial passages were made by intracerebral inoculations but other routes have been studied to some extent. In early generations attempts to produce an infection by inoculation of the chorio-allantoic membrane and by intramuscular inoculation

strain "H," has been passed only three times but histological study of brains of embryos of the third generation revealed changes characteristic of rabies.

CLINICAL EVIDENCE OF RABIES IN INOCULATED EMBRYOS

Intracerebral inoculation resulted in death, presumably from trauma, in some of the embryos within 24 hours after inoculation but with proper technic this mortality should not exceed 30 per cent and it may be as low as 10 per cent. During the next 5 or 6 days, deaths were extremely uncommon. Some embryos died between the sixth and eighth days when they were 19 or 21 days old, and it is probable that these deaths were due to rabies; however, many, and in fact most, of the embryos survived considerably longer. Many embryos survived inoculation for 13 days and one lived for 14 days. Thus it is possible for an embryo to survive 5 or 6 days beyond the expected hatching date. Development was slowed to some extent but the embryos continued to exhibit rhythmic movements. In the embryos which went beyond the normal hatching date the yolk became inspissated and it was not incorporated within the peritoneal cavity. In my experience no embryo inoculated intracerebrally with rabies virus has ever hatched.

PATHOLOGICAL CHANGES ASSOCIATED WITH RABIES IN THE CHICK EMBRYO

No gross changes were observed until the third or fourth embryo generation when a mild hydrocephalus was noticed. This hydrocephalus, which was observed in each of the three strains, became much more severe in later generations. It was detectable as early as the third or fourth day and it increased rapidly after this time. The hydrocephalus was symmetrical and it was unassociated with any appreciable enlargement of the skull or thinning of the tables. The entire ventricular system was dilated and there was also an enlargement of the subarachnoidal spaces, especially those around the cerebellum. Symmetrical hemorrhagic necrosis of the tectum and of the forebrain appeared in later generations.

Microscopic examination revealed, in addition to the hydrocephalus, an hydromyelia. There were also encephalomalacia and

TABLE II
Inoculation of Other Host Species with Embryo-Adapted Virus

Strain C					Strain R				
Number of passages in embryo	Host		Route*	Results**	Number of passages in embryo	Host		Route*	Results**
	No.	Type				No.	Type		
3	6	mice	icer.	8, 8, 8, 8, 8.	4	6	rabbits	icer.	5, 7, 7, 7, 8.
17	5	mice	icer.	7, 7, 7, 7, 8.	14	3	dogs	icer.	5, 5, 5.
21	3	mice	icer.	7, 8, 9.					
	3	mice	sbc.	10, S, S.					
	3	mice	imus.	8, 10, S.					
22	6	rabbits	icer.	10, 13, 16, S, S, S.					
26	6	dogs	icer.	9, 9, 9, 10, 10, 10.					
29	13	rabbits	icer.	11, 11, 12, S, S, S, S, S, S, S, S.					
43	6	mice	icer.	7, 9, 10, 11, 12, 13.					
	3	dogs	icer.	9, 9, 14.					

* icer. = intracerebral; sbc. = subcutaneous; imus. = intramuscular.

** Figure indicates day of death. S indicates that the animal became sick but survived.

were unsuccessful. However, even in the earliest attempts (sixth generation) intra-ocular inoculation was always successful. In later generations infection was established by inoculations on the chorio-allantoic membrane, in the muscles of the thigh and in the yolk sac. Complete studies of the disease produced by these routes of inoculation have not yet been made but the end results appear to be the same regardless of the route of inoculation: hydrocephalus develops, Negri bodies are numerous and there is extensive destruction of nervous tissue. The incomplete studies of other routes indicate that the incubation period is somewhat longer when routes other than the intracerebral are used. The pathogenesis of the disease following these other routes of inoculation is being investigated.

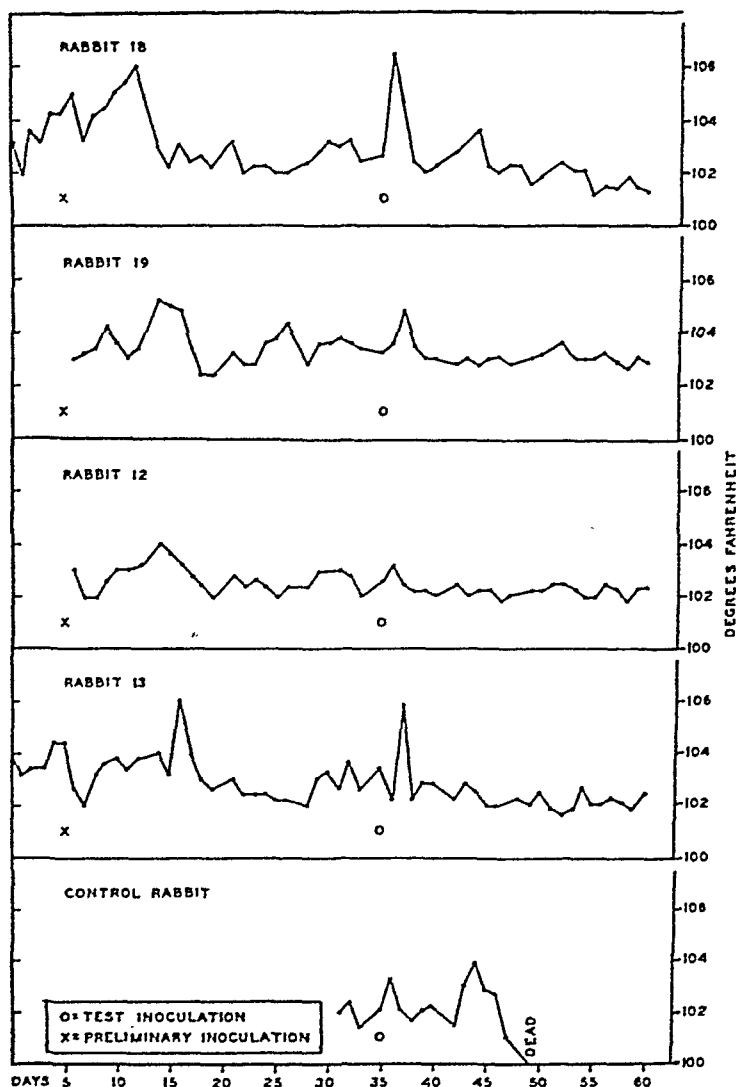
VIRULENCE OF CHICK-EMBRYO VIRUS FOR VARIOUS HOST SPECIES

Chick-embryo virus was inoculated into other species of animals in order to determine whether or not changes in its virulence for these hosts had occurred. These studies are summarized in Table II.

There are several points of interest which are not shown in this table. Strain C, which has been studied most carefully, was first reinoculated into mice after three passages in the embryo. These mice developed weakness and paralysis on the sixth or seventh day and died on the eighth day after inoculation. Sections from the brains revealed many Negri bodies and much perivascular exudate. From a clinical and pathological point of view these mice behaved as mice usually do when infected with street virus. Seventeenth generation virus was also put into mice. They became ill on the fifth or sixth day and died on the seventh or eighth day but they exhibited tremors and convulsions instead of weakness and paralysis. At autopsy very few Negri bodies were found and there was meager perivascular exudate. In this generation, therefore, there was definite evidence of alteration in the virus from a clinical as well as a pathological aspect.

Twenty-first generation virus was inoculated intracerebrally, subcutaneously and intramuscularly, using 3 mice in each group; all developed tremors and convulsions and 6 of the 9 died. However, 2 of the subcutaneously inoculated mice and 1 of those inoculated intramuscularly survived.

passage virus are shown in Text-Figure I. All of the test rabbits survived the inoculation, whereas the control animals receiving 0.5 cc. of 0.05 per cent emulsion died of rabies. The rise in temperature which occurred after the reinoculation of 3 of the test



TEXT-FIG. I.

Temperature charts of rabbits receiving the original and test inoculations, and of one control rabbit.

animals requires explanation. The animals, both test and control, were inoculated and within 15 minutes the temperatures were taken. A similar rise in temperature, immediately following intracerebral inoculation, has been noted in normal animals, so it seems likely that this fever is simply a reaction to trauma and not an immunity response.

Encouraged by these survivors, 22nd generation virus was inoculated intracerebrally into 6 rabbits and they developed fever, extreme ataxia and incoördination. Because of this they were unable to eat but if food was held for them they were able to chew and swallow it. However, no attempt was made to maintain their nutrition. One animal appeared prostrate on the tenth day but was not paralyzed. This animal was killed and sections of its brain showed a few Negri bodies and some exudate. One rabbit died on the 13th day and another on the 16th day. Histological examination of their brains revealed no Negri bodies and a 10 per cent emulsion of brain from the rabbit which died on the 16th day failed to produce rabies when inoculated intracerebrally in mice. The remaining animals gradually recovered except for some residual ataxia.

Twenty-sixth generation virus inoculated intracerebrally in dogs produced a uniformly fatal disease characterized by fever, tremors, ataxia and convulsions. Twenty-ninth generation virus inoculated intracerebrally in 13 rabbits produced symptoms of mild fever and ataxia in all of them, but only 3 died. Forty-third generation virus given to mice and dogs produced a fatal disease similar to that already described for these species.

Very little work has been done with strain R but it was highly virulent for dogs and rabbits, producing a rapidly fatal disease characterized by fever and flaccid paralysis.

IMMUNITY TESTS ON SURVIVORS

The 3 mice which survived inoculation of the 17th generation of strain C were tested for immunity by an intracerebral inoculation of 0.03 cc. of 10 per cent mouse-passage virus. This procedure was not followed by any detectable change in the test mice but control mice died in 8 days.

Four of the rabbits surviving the intracerebral inoculation of the 29th generation of strain C were also tested for immunity by the intracerebral inoculation of 0.5 cc. of 5 per cent emulsion of two strains of rabies virus: one was rabbit-fixed virus and the other was mouse-passage human virus. Temperature charts of the rabbits which received the original and test inoculation and of 1 control rabbit which received 0.5 cc. of 0.05 per cent mouse-

of the pathogenicity of this virus for the rabbit and the mouse. This alteration is of such magnitude that it is possible to produce a self-limited, nonfatal disease in rabbits by intracerebral injection and in mice by subcutaneous or intramuscular injection of embryo-passage virus. Furthermore, tests show conclusively that recovery from the mild disease is followed by the development of a solid immunity to intracerebral inoculation of rabbit-fixed virus and mouse-passage virus.

The immunizing properties of chick embryo virus are important from both a theoretical and a practical point of view. The development of an actively immunizing strain of rabies virus, whereby immunity resulting from actual infection may be produced, would make it possible to study the immune response of animals to the complete rabies antigen. Heretofore this has been impossible since the immune response to vaccines has been dependent upon the pre-formed antigen introduced during vaccination, and furthermore this antigen has been altered by the inactivation of most vaccines. From a practical point of view such a strain of virus would be an ideal vaccine since it would be possible to immunize the animal with a single dose of virus which actually produced a mild infection. It would, of course, be essential that the mild disease produced in this fashion be nontransmissible in nature.

At present the embryo-passage virus is still highly virulent for dogs but it is hoped that repeated passages in the embryo will bring about alterations in virulence for dogs comparable to those which have already occurred for rabbits.

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DISCUSSION

These experiments are important from at least two points of view.

1. They provide a new experimental host in which rabies may be studied. The chick embryo is a unique host in many respects but its chief value in the experimental study of rabies lies in its ability to withstand a massive inoculation of virus for as long as 14 days. Since evidences of infection of the central nervous system are present as soon as 3 days after inoculation, this means that the embryo has had rabies for about 11 days, during which time the greater portion of the nervous system has been destroyed. This remarkable ability of the embryo to withstand the infection much longer than other hosts makes it possible to study the more mature lesions of rabies. These mature lesions are remarkable because of their widespread distribution and their extremely destructive nature. Six days after intracerebral inoculation the lesions occur throughout the central nervous system and in the retina and it is difficult to find any peripheral ganglion which does not show evidence of infection. In addition there is extensive destruction of nerve tissue which is associated with the development of a marked hydrocephalus and hydromyelia as well as encephalomalacia and myelomalacia. It seems likely that the hydrocephalus is due, in part at least, to the destruction of undifferentiated elements which in normal development would have continued to multiply and differentiate to form the mature elements of the nervous system. The destruction of these immature, actively proliferating cells would undoubtedly have a tremendous effect upon subsequent development of the brain and cord. It is of course obvious that destruction of fully matured nerve cells is also important in the genesis of this lesion. It is unlikely that the hydrocephalus develops on the basis of obstruction: it did not occur in the first three or four generations; it has not been observed (except in extremely rare instances) when noninfectious brain was inoculated intracerebrally; it is not associated with enlargement of the skull; and it develops when virus is inoculated by routes other than intracerebral.

2. The experiments indicate that intracerebral passage of rabies virus in the chick embryo is associated with a profound alteration

DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Gross photographs of head of an embryo inoculated intracerebrally on the 13th day of incubation with 22nd generation of strain R. Killed 6 days after inoculation. The lower photograph of the dorsum of the skull shows an hydrocephalus of mild degree and in addition two black areas in the forebrain. These are areas of hemorrhagic necrosis. The upper photograph is a longitudinal section a few millimeters to the left of the midline. It shows the forebrain to be somewhat smaller than normal. The major portion of the forebrain is quite dark due to hemorrhagic necrosis. Immediately behind the forebrain is seen the moderately dilated lateral ventricle. Below this ventricle lies the tectum. It is not necrotic but its ventricle is greatly dilated.

FIG. 2. Gross photographs of the head of an embryo inoculated intracerebrally on the 13th day of incubation with 42nd generation of strain C. Killed 6 days after inoculation. The lower photograph shows the dorsal aspect of the skull. Note the dark portion which lies behind the small lobes of the forebrain. These are dilated ventricles. The upper photograph shows a longitudinal section about 3 mm. to the left of the midline. The small forebrain lies just above and a little behind the eye. Immediately behind is the greatly dilated ventricle. Below the ventricle lies the tectum, part of which is grayish in color and part black. The black portion is the site of hemorrhagic necrosis.

FIG. 3. Photomicrograph of section of embryo head in a plane 1 or 2 mm. to the right of the midline. Embryo inoculated intracerebrally with 45th generation of strain C. Killed 6 days after inoculation. The small forebrain lies above the eye. A thin strip of nervous tissue and meninges extends backward from the superior portion of the forebrain. This strip parallels the skull for a distance of about 15 mm. and then turns downward and finally forward again to merge with the base of the brain. Behind the small forebrain lies the greatly dilated lateral ventricle which is bounded above and behind by the thin strip of tissue already described. The portion of brain which extends downward and backward from the forebrain appears extremely pale. Only a thin shell of forebrain remains posteriorly, and immediately beneath this is seen the tentorium cerebelli. The cerebellum with its thin, simple convolutions surrounded by the greatly dilated subarachnoidal space is well illustrated. The fourth ventricle is greatly dilated. The choroid plexuses of the fourth ventricle and of the lateral ventricle are clearly shown. $\times 4$.

FIG. 4. Photomicrograph of head of normal embryo of the same age as the one in Figure 3. This section is taken in almost exactly the same plane as that in Figure 3 and is included for comparison. $\times 4$.

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PLATE 39

FIG. 5. Low power photomicrograph of cord and dorsal root ganglion from embryo inoculated intracerebrally with 17th generation of strain C. Killed at 6 days. Note the wide subarachnoidal space and the rarefied appearance of the cord itself. In the dorsal root ganglion are seen two large accumulations of round cells. $\times 40$.

FIG. 6. Photomicrograph of the same section used for Figure 3. The field photographed lies directly behind the eye. This photomicrograph shows the extreme degree of encephalomalacia. $\times 40$.

FIG. 7. Higher power photomicrograph of a field similar to that shown in Figure 6. Note the extreme rarefaction of the tissue. Blood vessels are numerous and a few cells persist around them, but the intervening tissue is almost completely destroyed. Inflammatory cells are notably few. $\times 65$.

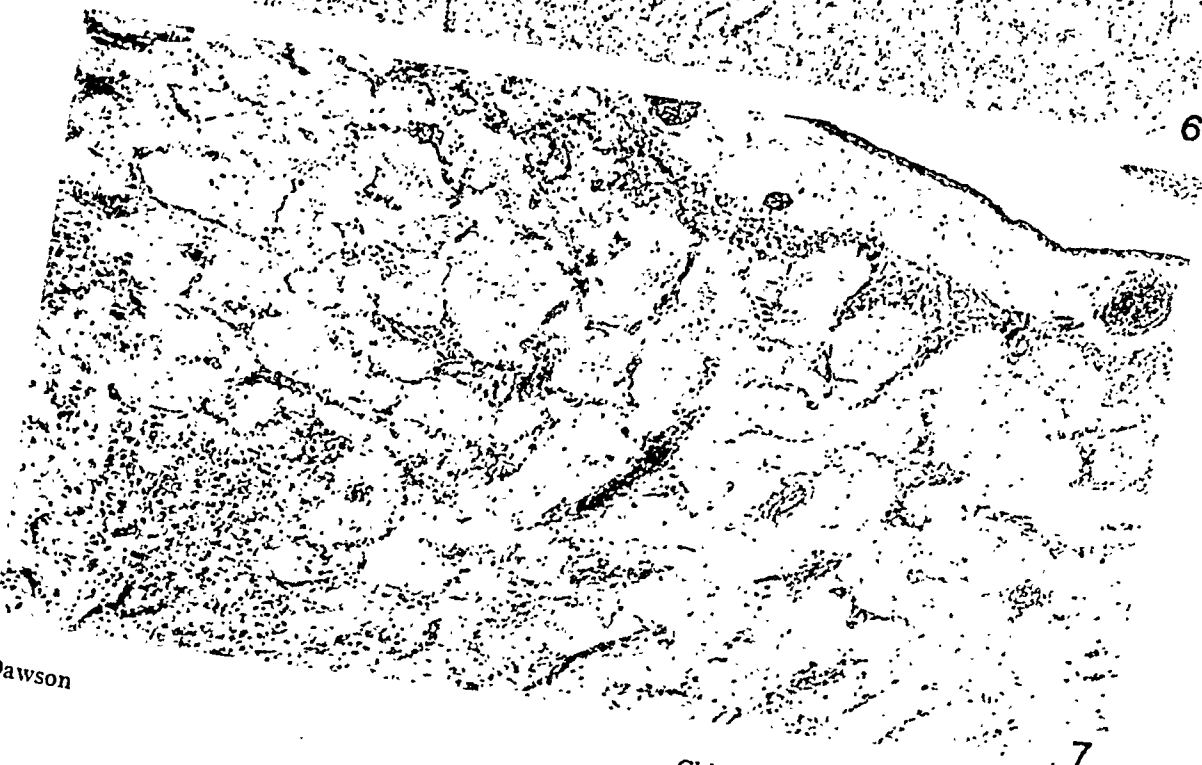


PLATE 40

FIG. 8. A photomicrograph of a ganglion just beneath the serosa of the gizzard of an embryo 6 days after an intramuscular injection of 60th generation of strain C. The large black masses are Negri bodies. There is also some necrosis of nerve cells and some lymphocytic infiltration of the ganglion. $\times 120$.

FIG. 9. Photomicrograph of small ganglion within the muscular layer of the gizzard of a chick embryo 6 days after an intracerebral inoculation of 17th generation of strain C. There are numerous Negri bodies within the cytoplasm of nerve cells. Halos surround several of the inclusions. There is no lymphocytic infiltration and very little necrosis. $\times 800$.

FIG. 10. Another ganglion from the gizzard of the same embryo showing a few Negri bodies in the cytoplasm of neurons and a rather massive infiltration of round cells. $\times 350$.



Dawson

Chick-Embryo-Adapted Rabies Virus

PLATE 41

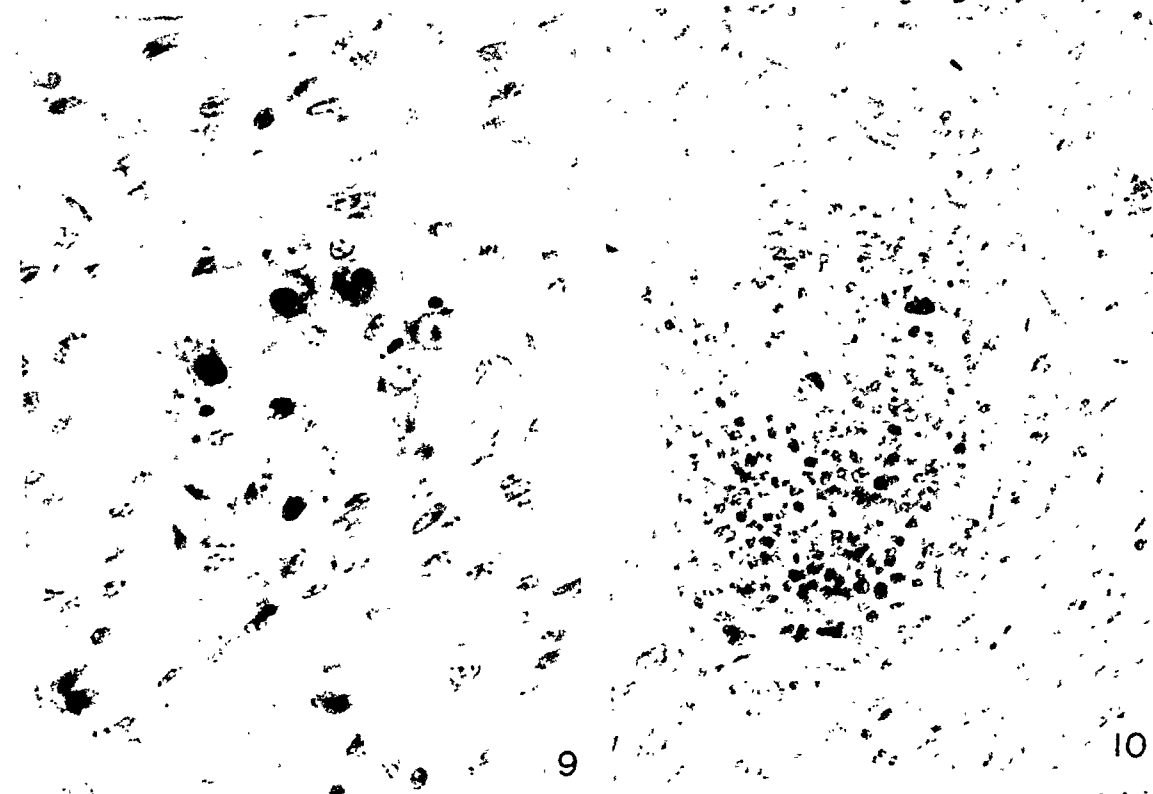
FIG. 11. Large Negri body in cytoplasm of cerebral neuron of chick embryo. $\times 1500$.

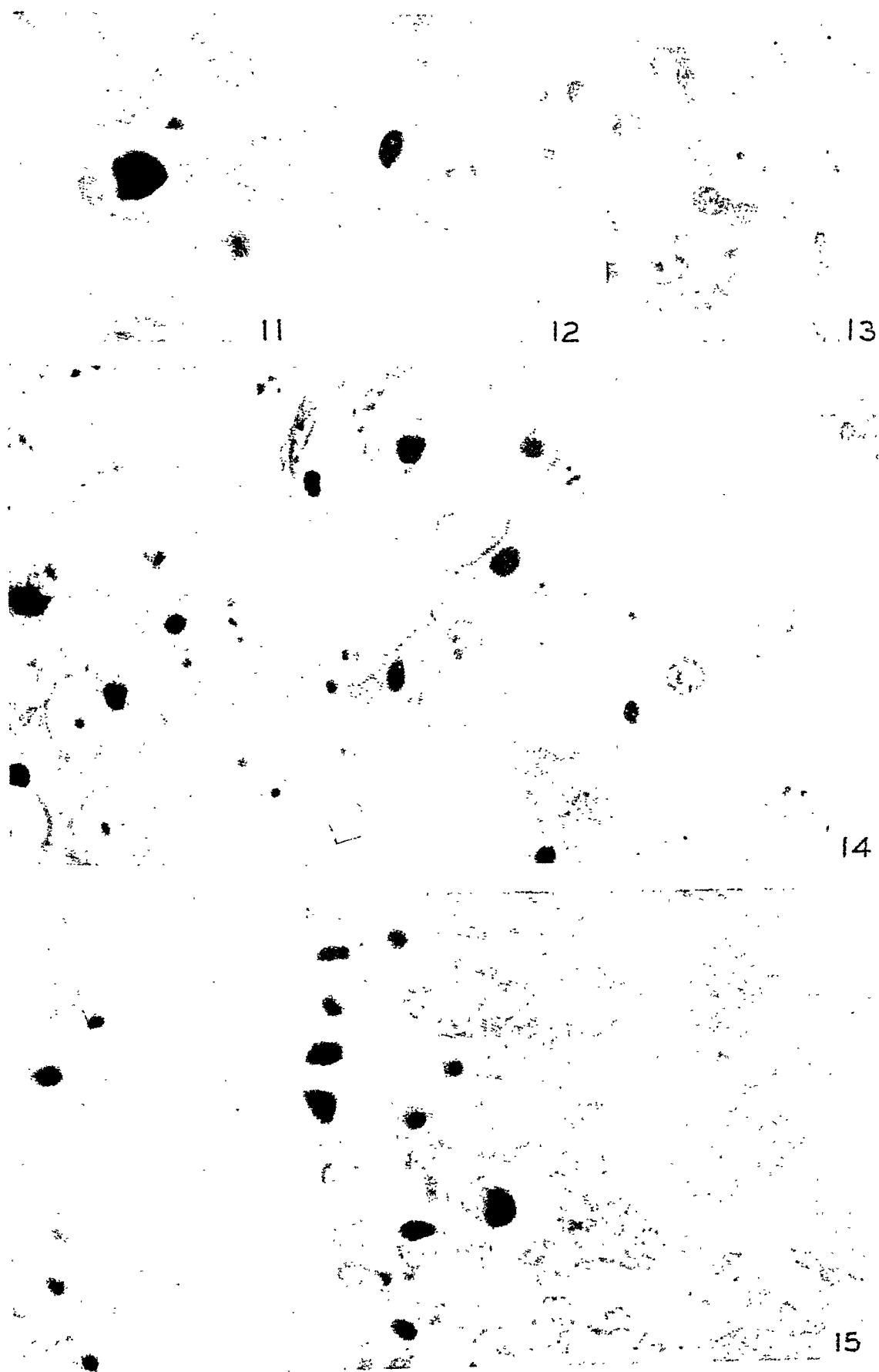
FIG. 12. Neuron in chick embryo brain showing vacuolated Negri body in cytoplasm. Carbol-aniline-fuchsin methylene blue. $\times 1500$.

FIG. 13. Negri body in cytoplasm in neuron of sympathetic ganglion of chick embryo. $\times 1500$.

FIG. 14. Several nerve cells which contain Negri bodies. $\times 1500$.

FIG. 15. Retina of chick embryo showing Negri bodies in all layers except the rods and cones. (Negri bodies occur in the rods and cones but they are not very numerous.) $\times 1500$.





MATERIAL AND METHODS

The mice which served in these investigations and which had received transplants of the anterior hypophysis were the ones which had been studied previously by Loeb and Kirtz⁴ as to the effect of these transplants on the growth and carcinomatous transformation of the mammary glands.

Transplants of the Anterior Lobe of the Hypophysis

Most of these experiments were carried out in virgin A mice, but also some mice of strains C57, C₃H, and CBA were used. Seventy-six mice, all females except two, received three or four, and in three instances five, transplants of the anterior lobe of the hypophysis of litter mates, irrespective of the sex of the latter. If the litters were too small, additional pituitary glands of unrelated mice of the same strain were transplanted. As a rule the mice were 1½ to 2 months old at the time of transplantation, but in four instances the mice had reached the age of 3 and 3½ months. The transplants were allowed to remain in the host for 1 and 2 weeks, and for 1, 2, 3, 5, 6, 8, 9, 10, 11, 12 and 14 months, at the end of which periods the animals were sacrificed (Table I).

TABLE I

The Duration of the Experiment, the Number of Transplanted Glands and the Number of Mice in the Various Strains

Duration of experiment	Number of mice	Strains									
		A			C57			C3H		CBA	
		No. of glands used			No. of glands used			Glands used		Glands used	
		3	4	5	3	4	5	3	4	3	4
1 week	5	2	1		1	1					
2 weeks	5	1	2		2						
1 month	6	3	1		2						
2 months	6	2	2		1	1					
3 months	6	3	1		1	1					
5 months	7	2	3		1	1					
6 months	3	1	1	1							
8 months	3	1	1					1			
9 months	14	4	2		2	2		1	1	1	1
10 months	4	2	2								
11 months	5	1	3	1							
12 months	4							2	2		
14 months	8	3			2	2	1				
Total	76	25	19	2	12	8	1	4	3	1	1

EFFECTS OF SYNGENESIOTRANSPLANTS AND OF EXTRACTS OF THE ANTERIOR LOBE OF THE BOVINE HYPOPHYSIS ON THE AGE CHANGES IN THE LONG BONES AND JOINTS OF MICE *

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In previous investigations we ^{1,2} have shown that implants and extracts of the bovine anterior pituitary gland (1) increase proliferation, (2) induce degenerative changes in the epiphyseal and articular cartilage, and (3) stimulate the deposition of bone in the skeleton of the guinea pig. Under apparently identical experimental conditions, either the processes of proliferation or those of degeneration and ossification of the cartilage predominated. Gigantism was not observed in connection with either mode of reaction. Yet, the predominance of proliferation caused a temporary retardation of skeletal ageing, whereas an acceleration was noted if degenerative changes and ossification were more pronounced than growth of cartilage. If the anterior hypophyseal hormone was administered to young animals, the tendency of the cartilage to proliferate was stronger than in older guinea pigs under corresponding conditions; conversely, the tendency of the cartilage to degenerate was the greater the older the animals were when the treatment was begun.³ But, the age factor could be only one of several which might be responsible for these different modes of reaction in the cartilage.

In a further attempt to clarify this problem, we analyzed the changes in bone and cartilage taking place under the influence of injections of extracts of the bovine anterior hypophysis as well as of homeo- and syngenesiotransplants of pituitary glands in closely inbred strains of mice.

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differences were found to exist in different strains of mice as to the intensity and rapidity with which these changes took place. In D mice they progressed at a faster rate than in C₃H mice, and in C₃H mice, more rapidly than in A mice. In C57 and CBA mice skeletal ageing proceeded more slowly than in either A, C₃H or D mice. Breeding also accelerated the course of the age changes in mice belonging to strain C57. In C₃H mice, and to a certain extent also in D mice, more bony tissue was present during the first two periods of skeletal development than in C57 or CBA mice. Furthermore, in old mice, hypertrophy and retrogressive changes in the articular cartilage could be observed, the incidence of these processes again being higher in A, D and C₃H mice than in C57 or CBA mice.

II. Effects of Syngenesiotransplants of the Anterior Lobe of the Hypophysis

A. Zone of Endochondral Ossification. In mice 7 to 12 weeks old, which had received transplants 1 or 2 weeks previous to autopsy, the growth zones were definitely narrower than in normal animals of the same strain and age (Figs. 1 and 3). This narrowing was due to a decrease in the number as well as in the size of the individual cartilage cells. In the epiphyseal disk of the upper tibia only 1 or 2 hypertrophic cartilage cells were present in one column instead of 2 or 3, as is normal for this age; the number of columnar cartilage cells was 5 or 6, which is slightly below the normal figure of 6 or 7. The cartilaginous ground substance was increased in amount and strongly calcified; its fibrils were thickened. The resting and the columnar cartilage cells showed less proliferation than normally, mitoses being scarce or absent. The largest hypertrophic cartilage cells were smaller than the corresponding cells under normal conditions. Thus, 1 or 2 weeks after transplantation and at an age of 8 to 9 weeks, the appearance of the epiphyseal disk of an A mouse was similar to that of an untreated A mouse 3 to 4 months old.

The subepiphyseal zone, in which the replacement of the cartilage by bone and the formation of the osseous trabeculae take place, became increasingly narrowed, and instead of well vascularized bone marrow, a poorly vascularized fibrous tissue was

Injections of Extract of Bovine Anterior Hypophysis

Thirty-three mice, thirty females and three males, belonging to strains C57, A, C₃H and D, received intraperitoneal injections of 0.1 or 0.2 cc. of freshly prepared bovine anterior hypophyseal extract on 4 or 5 days of the week. The age of the animals varied between 4 weeks and 2 months at the beginning of the injections, which were continued for periods of 1 and 2 weeks, and for 1, 3, 4, 6, 9, 10, 11, 13, 14, 15 and 16 months. The animals were killed 1 or 2 days following the last injection (Table II).

TABLE II

The Length of Time during which Injections of Anterior Pituitary Extract Were Made, and the Number of Mice of the Various Strains Used

Duration of experiment	Number of mice	Strains			
		C57	A	C ₃ H	D
1 week	4	2	2		
2 weeks	2		2		
1 month	2		2		
3 months	3	1	2		
4 months	2			1	1
6 months	2			2	
9 months	2			2	
10 months	1				1
11 months	4			2	2
13 months	2	2			
14 months	2	2			
15 months	5	4			1
16 months	2				2
Total	33	11	8	7	7

Specimens of the knee joint and dorso-ventral sections were prepared for microscopic study as described previously.⁵

MICROSCOPIC EXAMINATION

I. Normal Mice

The age changes taking place in the long bones during the life span of normal mice have been previously reported by us.⁵ We may restate briefly, that as far as these ageing processes are concerned, three periods could be distinguished: during the first, the predominant processes were proliferation of cartilage and apposition of osteoblasts; during the second, degeneration, calcification and ossification of the cartilage cells and matrix; and during the third, absorptive processes in the skeletal tissues. Remarkable

euhyaline cartilage remained. In the subepiphyseal zone, the resorption of the osseous trabeculae became more pronounced and the formation of the transverse osseous plate underneath the epiphyseal cartilage had set in and made progress. The bony lamella was thicker than in normal mice of any age and strain (Fig. 6). The condition of the epiphyseal plate in these mice was comparable to that of normal animals in the second year of life, but more bone had been laid down than in such mice and there was greater destruction of the cartilage. In mice of 11 months and older, in which the transplants had been allowed to remain for 9 or more months, the solution of the bony plate was accelerated, due to the increased activity of capillaries and other elements of the bone marrow advancing from the metaphysis in the direction towards the epiphysis. Thus, perforations of the ossified epiphyseal plate were initiated, which were at first narrow and became, subsequently, more or less widened. Thus a degree of epiphyseodiaphyseal union was produced, which was farther advanced than that of corresponding normal mice.

B. Joint. One or 2 weeks following transplantation the osseous border lamella, which separates the cartilaginous covering of the joint from the epiphyseal bone marrow, was thickened; the bone cells were small and their nuclei and cytoplasm were dense; the line of calcification which ordinarily traverses the zone of hypertrophic cartilage had advanced farther proximally into the transitional zone. One month after transplantation a softening of the cartilaginous matrix and an increased proliferation and enlargement of the cells of the transitional zone became noticeable. Two or 3 months following transplantation the ground substance was markedly swollen, the cells of the sliding and transitional zones had undergone hypertrophy and a pronounced amitotic and, sometimes, mitotic division. Simultaneously, pyknosis, karyorrhexis and karyolysis occurred in other cells of this area. Five months after transplantation and at still later periods, the proliferative and retrogressive changes had made farther progress (Fig. 9). An outgrowth of cartilage cells and a desquamation of liquefying hypertrophic cells into the cavity of the joint had occurred, leading to irregularities of the articular surface and, in places, to an exposure of the bone underneath the cartilage (Fig. 10). This bony tissue was subsequently absorbed and rarefied progressively

found in this layer. The calcareous spurs left over from the breakdown of the calcified cartilage capsules were covered by numerous actively proliferating epithelioid cells. The majority of these cells acted as osteoblasts, whereas only a few of them produced, by coalescence, osteoclastic giant cells. Thus, since more bony substance was deposited than was absorbed, the trabeculae were more numerous, thicker and more mature than is normal for this age and strain and they came to adjoin closely, with a broad base, the hypertrophic zone of the epiphyseal cartilage, whereas normally the formation of trabeculae takes place more distally in the metaphysis.

These conditions remained more or less stationary for the next 2 to 2½ months. In A mice, 5 months old, which had received anterior pituitary transplants at the age of 2 to 3 months, the epiphyseal growth zones had become still narrower (Fig. 5), the cell count having fallen to 1 or 2 hypertrophic and 4 or 5 columnar cells, whereas the corresponding normal figures are 2 or 3 hypertrophic and 6 or 7 columnar cartilage cells. Slight mitotic proliferation of the epiphyseal cartilage could also be observed, whereas proliferation of the epiphyseal cartilage ceases normally at the age of 4 months. Furthermore, marked retrogressive changes were not seen in the cartilage of the growth zones in bearers of pituitary transplants at this age, although they were present at this time in normal mice. Likewise, in transplant-bearing mice 6 months old the retrogressive changes were less pronounced than in normal mice at the age of 4 months. Consequently, the appearance of the epiphyseal disk of a mouse 5 months old which had received transplants was similar to that of a normal mouse 3 to 4 months of age. In the subepiphyseal zone, the production of bone around the trabeculae predominated over processes of resorption, and the formation of the transverse bony plate, which normally begins at the age of 4 months, was not seen in transplant-bearing mice 5 months old.

From 5 to 9 months subsequent to transplantation, the epiphyseal cartilage underwent an intensified and more rapid degeneration and calcification. Numerous amorphous plugs had taken the place of several disintegrated cartilage cell rows. These plugs subsequently underwent calcification or ossification, or both. Between these areas scanty remnants of sclerosed and calcified

III. Effects of Bovine Anterior Hypophyseal Extract

A. Zone of Endochondral Ossification. In mice 7 to 9 weeks old, after receiving injections of anterior hypophyseal extract for 1 to 2 weeks, the chondromucoid ground substance of the epiphyseal cartilage was loosened and swollen. The growth zones showed a regular columnar arrangement (Fig. 2). In the resting cells, and particularly in the columnar cartilage cells, mitoses were found more frequently than is normal for this age. The ordinarily flat columnar cartilage cells were enlarged and more vesicular, indicating their accelerated transformation into cartilage cells of the hypertrophic type. On the other hand, the layer of fully mature and intensely calcified hypertrophic cartilage cells was narrowed, as compared with the normal condition. This was due to the intensified breakdown of the hypertrophic cartilage cells caused by the rapid advance of capillaries from the bone marrow. The average number of hypertrophic cartilage cells in one cell column in the epiphyseal disk of the upper tibia had decreased from the normal number of 2 or 3, to 1 or 2, whereas the columnar cartilage cells, although they were enlarged and vesicular, were present in their normal number of 6 or 7.

After a series of injections extending over a period of 1 month, the cartilaginous matrix had become denser and more abundant and larger amounts of calcium had been laid down, particularly in and around the hypertrophic cartilage cells. The growth zones had become narrower than is normal for this age. In one cartilage cell column only 1 or 2 hypertrophic cells were counted, whereas the number of columnar cartilage cells had decreased to 5; still, the latter cells continued to proliferate, frequently by mitosis.

In these early stages the subepiphyseal zone contained many congested capillaries. Round epithelioid cells of the bone marrow underwent mitotic proliferation and arranged themselves in a beadlike manner along the calcareous spurs left over from the breakdown of calcified cartilage cell capsules. Subsequently, these osteoblasts became smaller; they were surrounded by osseous substance and were converted into osteocytes. Other epithelioid cells coalesced and formed osteoclasts, but these cells were not very numerous. After injections had been given for 1 month,

by capillaries growing out from the epiphyseal bone marrow and penetrating deeply into the bony lamella. Edematous swelling, as well as proliferative and degenerative changes in the cells, had involved also the capsule and the ligaments of the joint (Fig. 11). From these combinations of growth and retrogressive processes severe arthropathic lesions resulted. In mice in the second year of life, organization of degenerated areas took place through ingrowth of vascularized connective tissue.

C. Diaphysis. During the first months following transplantation the periosteal connective tissue was dense and it contained thickened collagenous fibers. The surface of the cortex was smoother than in normal mice of the same age. The number of osteoblastic epithelioid cells on the periosteal as well as on the endosteal surface of the compacta was greater than that of osteoclastic giant cells. Therefore, the apposition of bone was more accentuated than was the absorption. The vascular canals traversing the bony shaft were narrow, the osteocytes were small, there was a large amount of osseous substance, and the cementing substance between the osseous lamellae was intensely calcified. Thus the compacta was firmer, thicker and more mature than is normal at this age. From 2 to 5 months following transplantation, the vascular canals became enlarged as compared with the condition found at the earlier stages, while the osteoblasts were somewhat less numerous. There was now an increase in the intensity of the absorptive processes and the compact bone appeared, therefore, only slightly thickened as compared with normal conditions at this age. After 6 to 12 months, the vascular and lacunar resorption had made still farther progress, while the new production of bone had not progressed to the same extent. In the second year of life the processes of solution predominated definitely over those of apposition and consequently a thinning of the compact bone ensued which surpassed in degree that of normal control animals.

The bone marrow was rich in cells during the first year of life. Toward the end of the first year, however, there was an increase in the amount of fat tissue and fibrous connective tissue, noticeable particularly in the epiphyseal marrow. In untreated mice similar changes were found only in the second half of the second year of life.

complete epiphyseo-diaphyseal union in the femur (Fig. 7) as well as in the tibia was accomplished, a condition which had not been observed in normal mice of this strain even in the third year of life. In other instances, however, the epiphyseo-diaphyseal union had not made much progress beyond the stage present at about 1 year of age. But even here it was always farther advanced than in corresponding control animals.

B. Joint. As early as after 1 week of injections, but still more so after 2 weeks or 1 month, a marked proliferation of the cells of the sliding and transitional zones was seen in the majority of cases. The cells multiplied frequently by mitosis, but more commonly by amitotic division. They also increased in size, both cytoplasm and nucleus being enlarged. The more intense the growth processes had been, the more accentuated became the subsequent retrogressive changes in the cartilage cells of the transitional zone, as was indicated by the occurrence of pyknosis, karyorrhexis and karyolysis of such hypertrophic cartilage cells. Simultaneously, capillaries of the epiphyseal bone marrow advanced and corroded the bony border lamella which separates the cartilage of the joint from the epiphyseal bone marrow. These processes led to the appearance of minute arthropathic changes in the transitional zone of the articular cartilage, not noted at such an early age in normal mice irrespective of their strain. After preceding periods of injections extending over 3 to 4 months, the proliferation of the articular cartilage was less marked, but the retrogressive changes and particularly the replacement of the hypertrophic cartilage cells by bone had made progress. The bony border lamella, which had been thinned out at the earlier stages, was now greatly thickened.

When the injections were continued for periods of 10 months and longer, marked changes of the articular surface became noticeable. Active proliferation and hypertrophy of the cells of the sliding and transitional zones had set in in association with retrogressive changes (Fig. 8). The fibers of the cartilaginous matrix were teased apart and the hypertrophic cells were liquefied. Ulceration of the surface of the joint occurred, first near the insertions of the ligaments, and later spreading to other parts of the cartilaginous covering. Subsequently, processes of proliferation and retrogression affected also the ligaments and the capsule

some of the osteoblasts began to arrange themselves in a transverse line close to the layer of hypertrophic cartilage cells. This represented an initial stage in the formation of the transverse osseous plate which in untreated animals was not seen before the age of 4 months.

In mice 5 months old, after injections for 3 months (Fig. 4), the number of cartilage cells in one column and the width of the growth zones were practically unchanged as compared with conditions seen after 1 month of injections. However, the cartilaginous ground substance had become still more abundant, sclerosed and pre-osseous, causing a shrinkage of the columnar and hypertrophic cartilage cells. In some areas degenerative changes had affected whole cartilage cell rows, and amorphous masses were found in their place. The latter became calcified and ossified, and numerous fairly thick, partly osseous, partly calcified, plugs traversed the epiphyseal disk in the direction from the metaphysis toward the epiphysis. The condition was as far advanced as in normal mice of the same strain 10 to 12 months old.

In the subepiphyseal layer, solution processes now predominated, causing a gradual corrosion and thinning of the osseous plate which separated the epiphyseal cartilage from the diaphyseal bone marrow. The longitudinal trabeculae were similarly affected, and finally disappeared.

After 4 months of injections, and to a more marked degree after 6 months and later, the growth zones were more and more narrowed, the cartilaginous matrix was increasingly sclerosed and osseous, and the columnar arrangement of the densely calcified cartilage cells became indistinct. In all strains studied, calcification and bone formation were accelerated as compared with conditions in normal mice of corresponding age. Thus, in a D mouse 6 months old, which had received injections for a period of 4 months, the main body of the epiphyseal cartilage was replaced by bone; wide gaps in the epiphyseal disk indicated an advanced stage of epiphyseo-diaphyseal union which had progressed even farther than it had in noninjected D mice in the second year of life. Similarly, in C57 mice 15 to 19 months of age which had been injected for 13 to 15 months, all or at least the greater number of the cartilage cells had been ossified, and in the most advanced cases the epiphyseal plate had disappeared. Thus a

have normally a slow rate of skeletal ageing, responded more readily and more strongly to the hormonal stimulation than did mice of strains A, D and C₃H, which normally exhibit a faster rate of skeletal ageing. In a C57 mouse 4 months old, which had received anterior pituitary transplants at the age of 2 months, the age changes in cartilage and bone were farther advanced than in a corresponding A mouse. Similarly, a C57 mouse 5 months old, which had been injected for 3 months, showed comparatively more degeneration and ossification of the growth zones and more osseous substance than an A mouse of the same age under corresponding experimental conditions. Strain differences were also noticeable at late experimental stages. Of two transplant-bearing mice 15 months old, one belonging to strain C57 and the other to strain C₃H, it was the C57 mouse in which the age changes of the skeleton were farther advanced. The same difference was found in two mice 17 months old of strains C57 and D which had received injections of anterior hypophyseal extract for 15 months, the age changes of the skeleton of the C57 mouse being ahead of those of the D mouse. Thus, as far as skeletal ageing is concerned, the order found in various strains under normal conditions is not maintained and may even become reversed under the influence of the anterior pituitary hormones.

Both the epiphyseal cartilage and the cartilage of the joint showed similar strain differences in the reaction towards hormonal stimulation. It was possible, in the joint, to distinguish three grades of changes: grade I was characterized by a slight but definite proliferation of the cartilage; grade II by a moderate degree of both proliferative and retrogressive changes; and grade III by marked proliferative and retrogressive changes which had produced pre-arthritic or marked arthritic lesions (Figs. 8 to 11).

The figures in Table III demonstrate that under the influence of the pituitary hormones the age changes in the joints are always intensified as compared with the normal condition. This intensification is indicated by a higher incidence, by a greater severity and by an earlier onset of these changes. Both pituitary extracts and implants call forth articular lesions, but the extracts exert a stronger effect than the transplants. Strain differences in the production of articular lesions become apparent if the figures for strains C57 and CBA are compared with those for strain D. In

of the joint, where an edematous swelling of the intercellular substance was seen. Furthermore, desquamation of amorphous masses of greatly hypertrophied cartilage cells was noted at the periphery of the joint. More or less severe arthritic lesions resulted from these changes.

C. Diaphysis. When anterior hypophyseal extract was injected for periods of from 1 week to 1 month, the periosteal connective tissue became softened and an increased mitotic proliferation of the periosteal cells was noted. A much larger number of spindle cells than usual were converted into round epithelioid cells, which likewise not infrequently underwent mitotic division. These cells were either transformed into bone cells and laid down osseous substance, or they formed giant cells and acted as osteoclasts. The cells lining the inner surface of the shaft showed changes similar to those in the periosteum, but to a lesser extent. The vascular canals were enlarged and congested and the compact bone, therefore, was somewhat less dense than normally. When the extract was administered for periods of from 3 to 6 months, the proliferation of the periosteal cells decreased and a dense collagenous and fibrillar connective tissue was formed. The shaft at this stage became harder, denser and slightly thicker than is normal. After continuation of the injections up to 1 year the conditions remained about stationary. In the second year of life, the vascular and lacunar absorption of bone was intensified and led to a thinning of the bone which was more pronounced than ordinarily.

The bone marrow was rich in cells during the first year of life; in the second year the tendency to form fatty marrow and fibrous tissue was increased, particularly in the epiphysis. But even in mice 18 months old which had been injected for 16 months, the replacement of the hemopoietic marrow by fat tissue and fibrous connective tissue was only partial and localized.

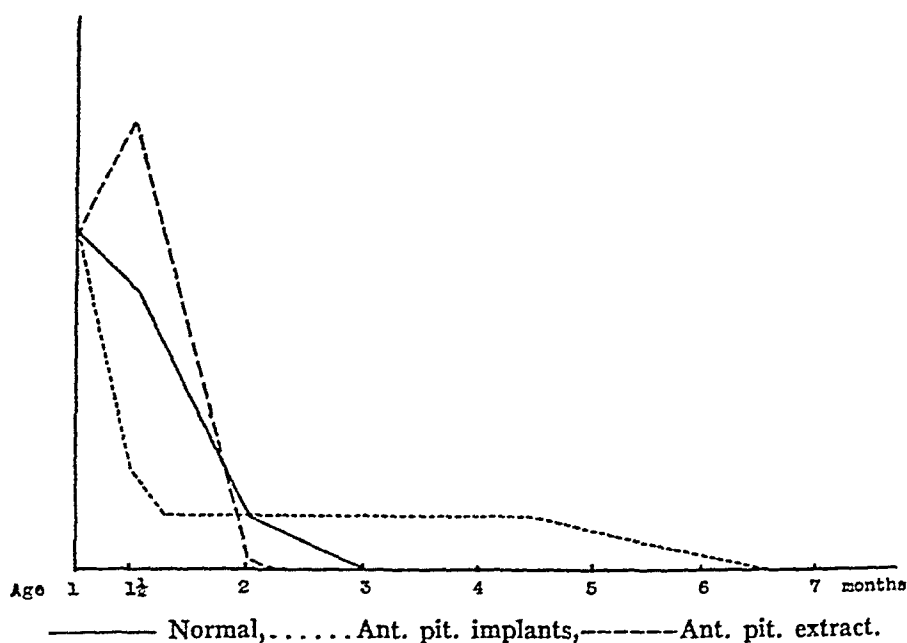
STRAIN DIFFERENCES IN THE RESPONSE TO THE STIMULATION BY ANTERIOR PITUITARY HORMONES

The degree of the reaction of the cartilage of the growth zones and joints after both implantation of hypophyseal glands and injections of extracts from cattle glands varied in different strains of mice. As a rule, mice belonging to strains C57 and CBA, which

Thus the course of the articular changes under the influence of stimulation by pituitary hormones is, to a certain extent, influenced by the strain to which the animal belongs. Mice exhibiting normally little tendency to develop articular age changes are more severely affected than those having a greater tendency to develop these changes spontaneously. The reaction of the articular cartilage, therefore, resembles that which takes place in the epiphyseal cartilage under the influence of the pituitary hormones.

COMPARISON OF THE EFFECTS OF SYNGENESIOTRANSPLANTS AND EXTRACTS OF BOVINE ANTERIOR HYPOPHYSIS ON CARTILAGE AND BONE

The following text-figures present the course of the proliferation of the cartilage of the growth zones, the degenerative changes in this cartilage and the formation and the absorption of bony



TEXT-FIG. 1. The proliferation of the cartilage of the growth zones.

tissue in this area and in the shafts of the long bones under normal and experimental conditions.

Text-Figure 1. The Proliferation of the Cartilage of the Growth Zones. In normal mice the degree of proliferation of the epiphyseal cartilage decreases gradually from the age of 1 month to the

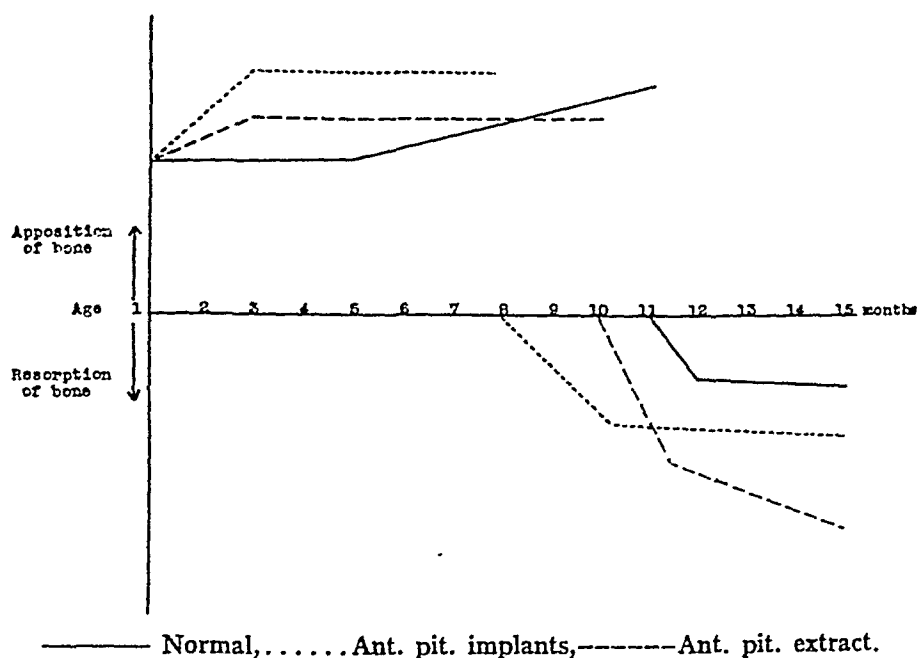
TABLE III
*The Distribution of the Degrees of Changes of the Articular
 Cartilage in Different Strains of Mice*

	Age	Experiment	Number of mice	Negative	Grade of changes*		
					I	II	III
Strains C57, CBA	mos.			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	1-5	Normal	15	100.0	0	0	0
	"	Transplant	10	56.7	33.3	10.0	0
	"	Extract	3	0	33.3	66.7	0
	6-18	Normal	33	42.4	30.3	18.2	9.1
	"	Transplant	13	15.3	30.8	30.8	23.1
Strain A	"	Extract	8	0	12.5	37.5	50.0
	1-5	Normal	9	100.0	0	0	0
	"	Transplant	14	42.8	21.4	35.8	0
	"	Extract	8	0	37.0	63.0	0
	6-14	Normal	10	10.0	50.0	40.0	0
	"	Transplant	32	9.4	25.0	40.6	25.0
Strain C ₃ H	6-12	Normal	6	16.7	66.6	16.7	0
	"	Transplant	7	0	28.5	43.0	28.5
	"	Extract	7	0	28.5	43.0	28.5
Strain D	6-18	Normal	10	30.0	20.0	20.0	30.0
	"	Extract	7	14.3	14.3	28.6	42.8

* The incidence of the changes taking place normally and under experimental conditions is given in percentages of the total number of mice used in each group.

both groups the experiments were continued until the animals had reached the age of 18 months. It is therefore possible to compare the figures obtained in these groups. In strains C57 and CBA, the normal incidence of the articular changes during the period from 6 months on is, for grades II and III, lower than in strain D. Under the influence of pituitary extract, however, strains C57 and CBA show a more marked increase in the articular changes than does strain D. No negative cases are recorded; cases with lesions of grade I show a diminution of 17.8 per cent, whereas those with lesions of grades II and III show an increase of 19.3 and 40.9 per cent respectively. Conversely, in strain D, in which the normal incidence of articular changes is much higher than in strains C57 and CBA, the increase due to the administration of the pituitary extract is relatively smaller than in strains C57 and CBA. The number of negative cases diminishes by 15.7 per cent; cases with lesions of grade I show a decrease of 5.7 per cent, and cases with lesions of grades II and III show an increase of only 8.6 and 12.8 per cent, respectively, as compared with the normal values for this strain.

peared at the age of about 4 months, or slightly later, and progressed steadily. Under the influence of the transplants the retrogressive processes began about 1 month later than in untreated animals, but they proceeded more rapidly and reached their maximum at the age of 10 to 11 months, which is earlier than normally. After administration of pituitary extract, the retrogressive changes were seen approximately 1 to 1½ months earlier than usual. During the first year, they made gradual progress and were always farther advanced than in normal animals.

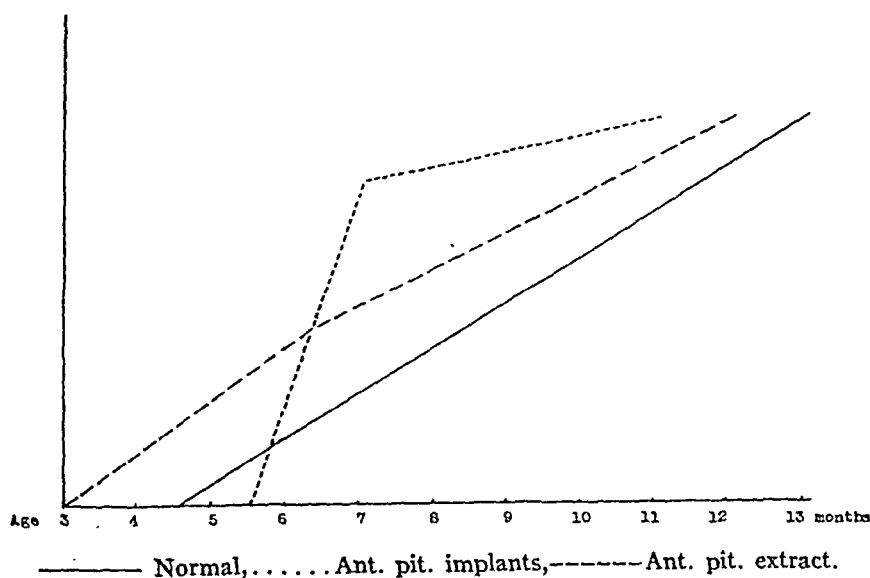


TEXT-FIG. 3. The formation and absorption of bony tissue.

Text-Figure 3. The Formation and Absorption of Bony Tissue.

In normal mice there was a fairly constant apposition of bone up to the end of the first year of life. In transplant-bearing mice, bone formation was increased over the normal during the first 8 months of life. This, in association with the increased degeneration shown in Text-Figure 2, led to a premature closure of the epiphyseal disk. At the age of 8 months, solution processes began to predominate; changes which were earlier and more intense than ordinarily. Subsequent to the administration of anterior pituitary extract, bone formation was increased over the normal degree, but less pronounced than after transplantation. Resorp-

age of 4 months, after which time it practically ceases. During the first 2 weeks subsequent to pituitary transplantation the proliferation of the epiphyseal cartilage decreases much more rapidly than under normal conditions. This causes the epiphyseal cartilage to have, at this time, the appearance of cartilage 1 to 2 months older than its actual age. Following this period of rapid decline the proliferation continues at this decreased level up to the age of 5 to 6 months, while normally proliferation ceases entirely at about the age of $3\frac{1}{2}$ to 4 months. Therefore, the epiphyseal cartilage looked younger at this stage than is normal. At the age of 7 months the proliferation of the cartilage in the animals bearing transplants had ceased. In mice receiving injections of pituitary extract, the proliferation of the epiphyseal cartilage was greatly increased during the first 2 weeks, after which period it declined very rapidly, but it was still above the normal level 4 weeks after the beginning of the injections. It then sank below normal and came to a standstill at about the



TEXT-FIG. 2. The degenerative changes in the cartilage of the growth zones.

age of 3 months. At the age of 3 months the epiphyseal cartilage had an appearance comparable to that of normal mice at the age of almost 4 months.

Text-Figure 2. The Degenerative Changes in the Cartilage of the Growth Zones. In normal mice the retrogressive changes ap-

by microscopical comparison of the epiphyseal cartilage of the experimental animals with that of normal animals of the same age.

In contradistinction to the anterior pituitary transplants, extract of the bovine anterior pituitary exerts, in the beginning, a temporary stimulation on the proliferation of cartilage. Subsequently, the growth period is shortened and thus compensation for the temporary overgrowth takes place and gigantism does not occur. Conditions in mice seem to resemble, therefore, those observed in guinea pigs² in which, after prolonged injections of anterior pituitary extract, there was likewise, following the transitory growth stimulation, a readjustment which prevented the occurrence of gigantism.

The difference in the response of the epiphyseal cartilage to the administration of the anterior pituitary hormone in diverse strains of mice may explain some variations which we² observed in the reaction of the cartilage in guinea pigs. Under apparently identical experimental conditions, proliferation of cartilage predominated over ossification in some animals, while the opposite condition was noted in others during the greater part of the experimental period. There are some indications that in this species also, at later stages, an adjustment takes place which restores the balance between the processes of proliferation and ossification. In the present experiments in mice the time of observation was much longer than in our earlier investigations in guinea pigs, the former being about $1\frac{1}{2}$ years as compared with 6 months in guinea pigs. Furthermore, $1\frac{1}{2}$ years in the life of a mouse corresponds to a much longer period in the life of a guinea pig. In view of the fact that mice of some strains react with a more rapid proliferation which is followed by a more marked degeneration and ossification, it may be suggested that in the guinea pig, also, similar strain differences may exist and that such differences may be responsible for the varying response of the cartilage to stimulation by the pituitary hormone in different individuals.

The microscopic observations recorded by us are in agreement with the statements by Allen⁹⁻¹¹ and Smith⁸ that in animals which have a sufficient supply of anterior pituitary hormone, an additional amount of this hormone cannot produce a permanent in-

tive processes set in at an earlier date than in untreated animals, but later than after syngenesiotransplantation of pituitary glands.

We may then conclude that both syngenesiotransplants and extracts of the bovine anterior pituitary accelerate the skeletal ageing of mice. However, there are some differences in the action of these two substances. The extracts call forth an initial brief rise in the proliferation of the epiphyseal cartilage which is followed by an accelerated retrogression. The primary effect of transplants is a decrease of the growth of cartilage, associated with a prolongation of the growth period and followed by an even greater acceleration of the degenerative processes. In addition, there is a further difference in the degree of the changes induced by transplants on the one hand, and pituitary extract on the other. More bony substance is found after transplantation than after administration of the extract, and, at later stages, resorptive processes are more marked in injected animals than in transplant-bearing mice.

DISCUSSION

Syngenesiotransplants of the anterior lobe of the hypophysis of mice survive and, in the mammary gland, increase the incidence of cancer (Loeb and Kirtz⁴). But in bone and cartilage, syngenesiotransplants of the anterior pituitary gland, instead of increasing the proliferation of cartilage, cause at first a definite inhibition of growth which lasts for some time. Subsequently, this deficiency of growth is balanced by a prolongation of the period during which proliferation takes place. Suppression of growth was likewise observed in urodele larvae which had received homeotransplants of embryonal hypophysis (Blount^{6,7}), and no growth promotion was obtained in similar experiments on anuran larvae by Smith,⁸ Allen,⁹⁻¹¹ and Burns.¹² On the other hand, increased lengthwise growth of the long bones of rabbits subsequent to homeotransplantation of whole pituitary glands has been reported by Pereira.¹³ No changes were noted in the epiphyseal disks of these rabbits, but the increase in growth was believed to have been due to a prolongation of the growth period. However, an extension of the growth period can be diagnosed only when there is an abnormal persistence of growth of the cartilage, and it should be possible to recognize such a condition

It is therefore possible that the transplants of anterior hypophysis act in two ways on cartilage and bone: they may act directly; or they may induce a greater production of estrogen and secondarily affect bone and cartilage by way of this hormone. Investigations in spayed mice are being conducted to determine whether an estrogen plays any rôle in the effect of pituitary transplants on the skeleton.

As to the effect of the anterior pituitary extract on cartilage and bone, this is present in thyroidectomized as well as in ovariectomized guinea pigs,^{19,20} and in thyroidectomized salamanders (Richardson^{21,22}). It appears probable, therefore, that also in mice the pituitary extract may act directly on the skeletal tissues.

SUMMARY

Anterior hypophyseal hormone exerts in mice (1) primary effects of a transitory nature on the growth of the epiphyseal cartilage and (2) permanent effects on cartilage and bone. The primary effects differ depending upon whether they are due to syngenesiotransplants or to intraperitoneally injected bovine hypophyseal extract. Under the influence of the former, the proliferation of the epiphyseal cartilage is at first inhibited, and this inhibition is followed by a prolongation of the period during which growth takes place. Bovine anterior pituitary extract causes at first a stimulation of proliferation of the epiphyseal cartilage, which is followed by a sharp decline and by a diminution in the duration of the growth period. The later and permanent effect of both syngenesiotransplants and bovine anterior hypophyseal extract is an acceleration of skeletal ageing. These changes lead to an accelerated epiphyseo-diaphyseal union, a premature absorption of the bony tissue of the shaft of the long bones, and an increase in the incidence and degree of arthropathic lesions. Strain differences determine, to a certain degree, the response of the skeletal tissues to the anterior hypophyseal hormones; mice of strains C57 and CBA reacting more intensely than those of strains A, C₃H and D.

NOTE: We wish to express our indebtedness to Leo Loeb for his advice in this study and to S. J. Hayward for the photomicrographs.

crease of growth beyond the normal degree. The primary effects which we have observed; namely, a diminution of the proliferative rate of the cartilage in animals with transplants and a primary stimulation in the case of extract, are temporary and are subsequently neutralized by a balancing mechanism.

Transplants and extracts of anterior hypophysis exert in addition, however, an effect on the skeleton which is permanent and which is not neutralized. This common effect is an acceleration of the normal processes of ageing which occurs simultaneously in various parts of the skeleton. In the growth zones of the long bones the epiphyseo-diaphyseal union is accelerated; in the shaft, senile absorption sets in prematurely; and in the joints, arthritic lesions are increased or prematurely initiated. In other animals anterior hypophyseal preparations have also been shown to cause an acceleration of development and ageing. Swingle¹⁴ and Allen⁹⁻¹¹ observed in frog larvae, and Clements and Howes¹⁵ in axolotl, an acceleration of metamorphosis under the influence of anterior pituitary hormone. Downs¹⁶ obtained a premature shedding of the deciduous teeth and precocious eruption of the permanent teeth in dogs subsequent to the administration of anterior pituitary hormone.

The question arises as to whether the anterior pituitary hormone induces the skeletal changes directly or by the intermediation of other endocrines. In mice, pituitary transplants do not exhibit a very great thyrotropic activity, but they exert a marked gonadotropic effect (Loeb and Kirtz⁴). Since, under the influence of the pituitary transplants, the output of estrogen is perhaps accelerated or increased, the changes in the long bones might possibly be due to this increase in the amount of estrogen. Previously we⁵ have shown that in normal mice, skeletal ageing proceeds more rapidly in strains with high mammary cancer incidence, and in breeding females. Furthermore, a similarity exists in the microscopic changes taking place in cartilage and bone of mice bearing transplants and in guinea pigs treated with estrogen.^{17,18} In both cases the early effect is an inhibition of proliferation of cartilage. This early reaction is followed by a second, characterized by a relative increase in proliferation, and by a third, characterized by an intense degeneration and ossification of cartilage.

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DESCRIPTION OF PLATES

PLATE 42

- FIG. 1. Section through the growth zone of the upper tibia of a normal virgin mouse of strain A, 8 weeks old. Epiphyseal line is wide and open. In the subepiphyseal zone, which is well vascularized, there is formation of trabeculae. $\times 150$.
- FIG. 2. Section through the growth zone of the upper tibia of a virgin mouse of strain A, which, beginning at the age of 7 weeks, had received injections of bovine anterior pituitary extract for 1 week. Many columnar cartilage cells are vesicular and the number of large hypertrophic cartilage cells is decreased. In the subepiphyseal zone the trabeculae are more numerous, longer and thicker than in Figure 1, their base being close to the zone of hypertrophic cartilage. $\times 150$.
- FIG. 3. Section through the growth zone of the upper tibia of a virgin A mouse which, at the age of $7\frac{1}{2}$ weeks, had received four syngenesio-transplants of the anterior lobe of the hypophysis and which had been sacrificed 1 week subsequent to transplantation. The growth zone is narrowed and partly calcified. The cartilage cells are decreased in size. The subepiphyseal zone is poorly vascularized. Thick and dense trabeculae reach up to the epiphyseal cartilage more closely than in Figures 1 and 2. $\times 150$.

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PLATE 43

FIG. 4. Section through the growth zone of the upper tibia of a virgin C57 mouse which, from the age of 2 months on, had received injections of bovine anterior pituitary extract for a period of 3 months. The cartilaginous ground substance is increased and hyalinized and numerous thick plugs have taken the place of disintegrated cartilage cell rows. There is formation of a transverse bony plate underneath the epiphyseal cartilage. $\times 150$.

FIG. 5. Section through the growth zone of the upper tibia of a virgin A mouse 5 months old which had received four syngenesiotransplants at the age of 2 months. There is no degeneration of the epiphyseal cartilage visible; the conditions in the epiphyseal cartilage are similar to those shown in Figure 3. $\times 150$.

FIG. 6. Section through the growth zone of the upper tibia of a virgin A mouse $10\frac{1}{2}$ months old which had received four syngenesiotransplants at the age of $1\frac{1}{2}$ months. The epiphyseal cartilage is almost completely ossified and only scanty remnants of hyalinized cartilage are left. The cartilage is enclosed on both sides by a thick bony plate. In the center of the disk advancing bone marrow is seen. $\times 150$.

FIG. 7. Section through the lower femur of a male C57 mouse $17\frac{1}{2}$ months old which, from the age of $2\frac{1}{2}$ months, had been injected with bovine anterior pituitary extract. The epiphyseal disk has been resorbed and complete epiphyseo-diaphyseal union has taken place. $\times 100$.



PLATE 44

FIG. 8. Section through the articular surface of the knee joint of a male D mouse 12½ months old which had been injected with bovine anterior pituitary extract for 10 months. Hyperplasia and hypertrophy of the articular cartilage are present. (Changes: grade I.) × 220.

FIG. 9. Section through the articular surface of the knee joint of a virgin A mouse 11 months old which had received four syngenesiotransplants of anterior pituitary at the age of 4 weeks. Moderate hyperplasia and hypertrophic growth of the articular cartilage are present near the insertion of a ligament. In some places, cells show degenerative changes; the cartilaginous covering is in process of solution. (Changes: grade II.) × 220.

FIG. 10. Section through the articular surface of the knee joint of a virgin A mouse 14 months old which had received five syngenesiotransplants of anterior pituitary at the age of 3 months. There is an outgrowth of hypertrophic cartilage near the insertion of the capsule of the joint. (Changes: grade III.) × 220.

FIG. 11. Section through the articular surface of the knee joint of a virgin A mouse 12½ months old which had received four syngenesiotransplants of the anterior pituitary at the age of 1½ months. Marked proliferation and degeneration of the articular cartilage are present. Ligaments show an edematous swelling and degeneration of cells. (Changes: grade III.) × 220.





MATERIAL AND METHODS

Normal spinal and gasserian (semilunar) ganglia of 7 white leghorn chickens, 5 white rats, 10 adult cats and 3 rhesus monkeys were perfused with a neutral 4 per cent solution of formaldehyde and dissected for study. In addition, 57 human gasserian ganglia and 7 spinal ganglia from cervical, thoracic and lumbar levels were obtained at autopsy. The sources of the human specimens ranged from the newborn to 81 years of age and specimens were selected regardless of sex, clinical symptoms or autopsy findings. Celiac and lumbar sympathetic ganglia were obtained from 8 human bodies for purposes of comparison.

Fixation was in ammoniated absolute alcohol, 10 per cent alcoholic chloral hydrate, or neutral 4 per cent solution of formaldehyde. Following fixation the tissues were carried through the Ranson pyridine-silver, and the Cajal silver impregnation methods. Sections of silver preparations were cut in a cranio-caudal plane at 12 μ . The formaldehyde-fixed specimens were cut at 8, 10 and 12 μ and alternate sections stained by the Bodian protargol and cresyl-violet technics. Serial sections of each specimen were examined, using an oil immersion lens as needed.

OBSERVATIONS

Earlier investigations concerning structural changes in sensory neurons concomitant with senility in the chicken and man have been reported by me.^{14,15} During the course of these studies the atypical and degenerate neurons have constantly aroused interest, not only because of their numerous and complexly ramifying processes, but also because of their marked resemblance to true multipolar neurons. Such similarity is demonstrated by sensory cells possessing supernumerary processes, commonly designated as "frayed cells of Cajal." These alone are considered in this investigation.

It would not seem possible, to one examining the sensory ganglia for the first time, that a normal unipolar neuron (Fig. 1) could undergo extensive transfiguration and thereby be confused with the structure of a multipolar neuron (Fig. 2). Yet, if one compares the appearances of the degenerate sensory cells in Figures 3, 6, 9, 12, 15 and 18 with those of sympathetic neurons in

DEGENERATE VERSUS MULTIPOLAR NEURONS IN SENSORY GANGLIA *

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Evidence has been presented for (Horton-Smith;¹ Kuré *et al.*;^{2,3,4} Okelberry;^{5,6} and Kahr and Sheehan⁷) and against (Duncan and Crocker;⁸ and Hinsey^{9,10}) the existence of spinal parasympathetic nerve fibers. Such attempts to challenge the Bell-Magendie law have been based on experimental degeneration of dorsal root fibers following root resection and on morphological studies of spinal ganglia under normal and experimental conditions.

Westbrook and Tower¹¹ have recently thrown new light upon the problem in their description of extraneous fibers which grow into the dorsal root following root resection and ganglionectomy. A detailed discussion of the recent literature can be found in their report.

The existence of parasympathetic fibers in the dorsal roots of spinal nerves would necessitate the presence of efferent (multipolar) neurons in either the spinal ganglia or within the spinal cord. Kuré, Saégusa, Kawaguchi and Shiraishi³ have described such cells of intraspinal origin as lying in the ventral portion of the dorsal horn, while Kiss¹² has described small, dark multipolar neurons in the spinal ganglia following prolonged osmification. Fisher and Ranson¹³ concluded that the stellate appearances of the dark cells described by Kiss were the result of cell injury and shrinkage.

In view of the above conflicting reports, a more extensive investigation with appropriate silver methods seemed desirable. By studying the finer ramifications of neuron processes in normal, senile and pathologic sensory ganglia one might expect to observe true multipolar neurons, should they exist.

* Received for publication July 30, 1940.

as definite axon or dendrite radicles. They possess few or no club-shaped outgrowths or reticulated expansions. Branching of the true dendrites is more uniform and equal.

In sympathetic ganglia from older specimens, both animal and human, one frequently observes a definite thickening or hypertrophy of neuronic processes (Figs. 5 and 14). In other instances all of the dendritic processes do not penetrate the capsule (Figs. 11, 13, 14, 17, 19 and 20). As a result of these inconsistencies, the contrasting features as listed may be less clearly defined.

Serial examination of all the animal and human specimens revealed no cell which could be classified morphologically as a true multipolar neuron.

Distribution of Degenerate Neurons. Spinal and gasserian ganglia of the chicken, rat, cat, monkey and man possess the described degenerate neurons in variable numbers. Though relatively few in the spinal ganglia of the lower animals, they are more frequent in the animal gasserian and human spinal ganglia. Festooned sensory cells are abundant in human gasserian ganglia from patients of all ages. Their occurrence is particularly frequent following death from diabetes, chronic alcoholism, barbitol poisoning and prolonged infectious diseases.

COMMENT

Embryologically, it would be possible to account for the presence of multipolar neurons in the sensory ganglia since the primordia of the spinal ganglia lie along the course of migrating neuroblasts destined for the sympathetic ganglia. Decision whether such neuroblasts are derivatives of the neural crest, neural tube, or both, awaits further investigation. In any case the opportunity presents itself at this early stage for migrating neuroblasts destined to become efferent neurons to be retained within the definitive spinal ganglia. Subsequent differentiation of the retained neuroblasts into multipolar neurons, which were later reached by preganglionic fibers, might then offer a plausible explanation for the presence of so-called spinal parasympathetic fibers in the dorsal root of spinal nerves.

In a similar way the gasserian ganglion might be expected to manifest such retained neuroblasts, since the primordia of the ciliary, submaxillary, sublingual and sphenopalatine ganglia are

Figures 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19 and 20, differentiation between the two cell types becomes progressively more difficult.

There appears to be no limitation on the number of such accessory processes of a unipolar cell, or on the complexity which each process may attain. With the addition of each new process, originating from either cell soma or undivided stem (axon), the sensory neuron more closely resembles the multipolar type.

In Figure 3 the accessory processes arise only from the axonic stump, and sensory neurons with this structure bear a likeness to the truncated multipolar cells of the sympathetic ganglia (Figs. 4 and 5). Sensory neurons demonstrating only a few short, thick radicles (Fig. 6) are similar to efferent neurons which possess a like number of processes (Figs. 7 and 8).

The degenerate unipolar cells in Figures 9, 12 and 15 are very common in senile and pathologic sensory ganglia of man. Due to the increase of processes and collateral branching, such neurons are easily confused with diverse sympathetic cells of closely parallel structure (Figs. 10, 11, 13, 14, 16 and 17).

Excessive proliferation of cytoplasmic outgrowths and cell excrescences results in the bizarre structure shown in Figure 18. The dichotomous branchings and profuse ramifications on these sensory neurons might readily lead one to assume that he was observing a motor neuron. The distinction is at times quite difficult in view of the similar structure and branching of the multipolar cells (Fig. 19). In addition, the so-called "comet-cells" of the sympathetic nervous system (Fig. 20) manifest a wealth of short branching processes, whereby they too simulate degenerate sensory components like those shown in Figure 18.

The features distinguishing between degenerate sensory and true multipolar neurons are shown in the accompanying figures. The short accessory processes of the sensory cell usually terminate within the limits of the surrounding capsule. The frequency of branching, the varicosities, and the terminal club-shaped expansions along the processes provide additional distinguishing criteria. Likewise, the abortive cell outgrowths stain more darkly with the usual silver impregnation methods than does the normal undivided stem of the sensory neuron.

In contrast to these features, the more lightly stained processes of the efferent cells pass through the confines of the cell capsule

the spinal or gasserian ganglion. The reality of preganglionic efferent fibers terminating in spinal ganglia remains questionable.

Degenerate sensory neurons often simulate multipolar cells. These two cell types may be distinguished from each other by the length, branching, varicosities and staining of their respective processes.

Osmic acid and Nissl technics are inadequate methods with which to classify nerve cell types, due to structural variations of sensory neurons in senile and pathologic ganglia.

Recognition of cell types and variation in the appearances of degenerate neurons is essential for correct interpretation of sensory ganglionic structure.

NOTE: The author wishes to express his appreciation to S. R. Detwiler and A. T. Rasmussen for their coöperation and helpful criticism throughout this investigation. He is indebted to the Departments of Pathology of the University of Minnesota and of the College of Physicians and Surgeons of Columbia University for aid in obtaining fresh specimens and clinical data. The author expresses his thanks to Julius Bloom for valuable assistance in the preparation of photomicrographs.

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known to arise in close association with branches of the trigeminal nerve (Streeter,¹⁶ Stewart¹⁷).

This assumption or retention theory is not wholly free from justified criticism. However, it affords a legitimate explanation for, and understanding of the significance of, multipolar neurons should they be found to exist in sensory ganglia. From the study of serial silver preparations of spinal and gasserian ganglia designed to demonstrate such cells, no morphological evidence could be found to substantiate their existence. In the absence of efferent neurons within the sensory ganglia, the reality of preganglionic efferent fibers in the dorsal spinal root is questionable.

The presence of many degenerate sensory neurons, designated by Cajal¹⁸ as "frayed cells" in view of their ragged and cog-wheel appearance, might readily lead to confusion of neuron cell types. In addition, terminology becomes confusing if investigators classify ganglion cells with supernumerary processes as multipolar. Such cells are multipolar only in the sense that they possess more than two processes and in these instances the term cannot be applied with the intention of denoting a motor or efferent physiological nature.

It should be pointed out that no technic can be utilized in determining the multipolarity of a ganglion cell unless it is capable of staining the individual processes and demonstrating their capsular and extracapsular relationship. The classification of neuron cell types following the osmification method described by Kiss¹² has been found unreliable, as was pointed out by Fisher and Ranson¹³ and more recently by Levi and Meyer.¹⁹

Neurons herein described as atypical must be looked upon as degenerate cells. Due to clumping of the cytoplasmic tigroid bodies in senile sensory cells (Truex¹⁵), the cresyl-violet and Nissl technics cannot be relied upon to distinguish adequately between all sensory and motor neurons.

SUMMARY

Serial silver preparations of the spinal and gasserian ganglia of the chicken, white rat, cat, monkey and man fail to demonstrate the presence of multipolar neurons. No morphological evidence has been presented to date which conclusively substantiates the existence of such neurons of parasympathetic function within

DESCRIPTION OF PLATES

PLATE 45

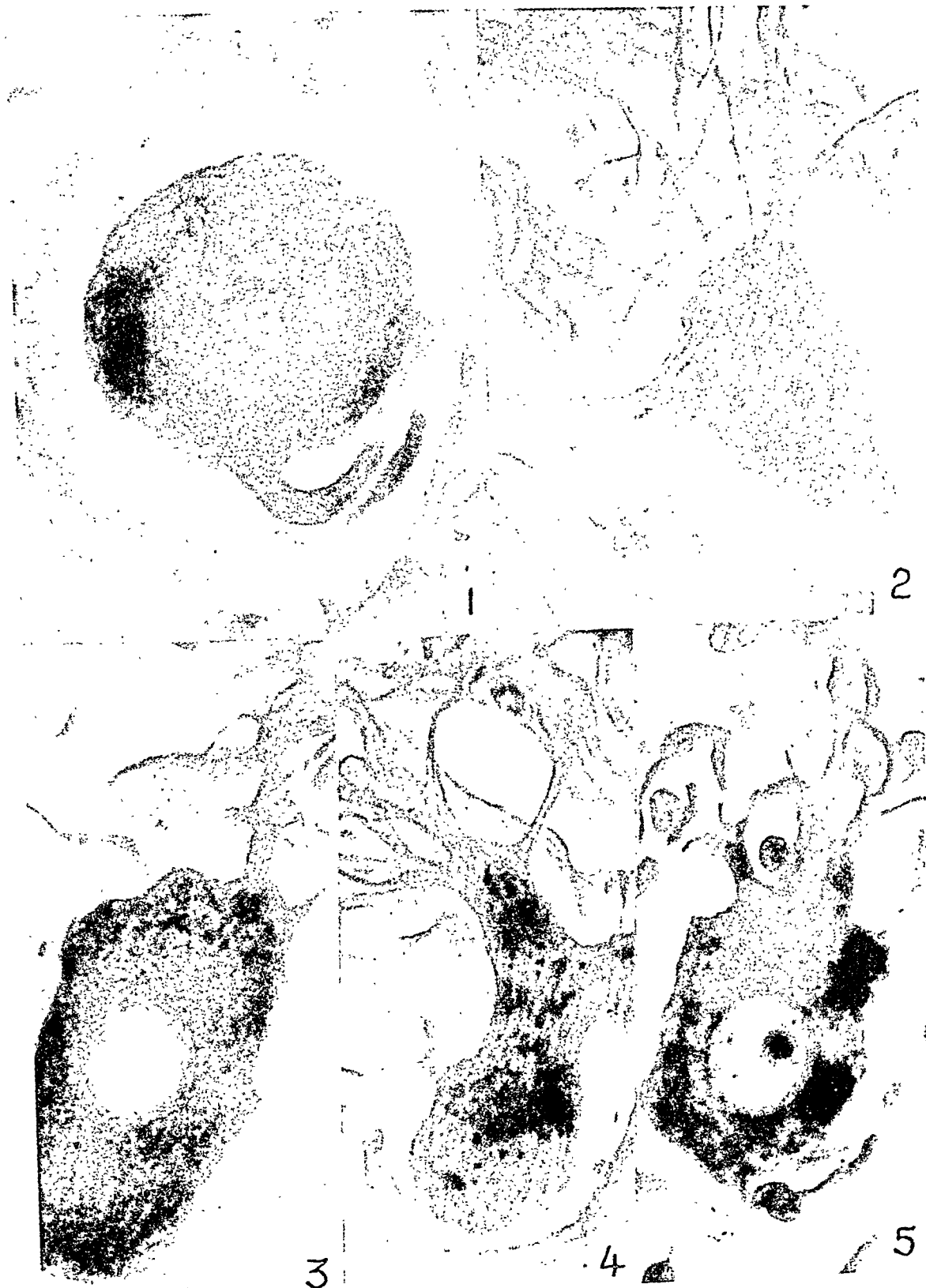
All figures are untouched photomicrographs from silver preparations of human material.

- FIG. 1. Normal unipolar neuron with capsule and process (axon). Gasserian ganglion. Male, 2½ years old. Cajal's technic. $\times 1000$.
- FIG. 2. Typical multipolar neuron with processes. Celiac ganglion. Female, 42 years old. Cajal's technic. $\times 1000$.
- FIG. 3. Degenerate unipolar neuron showing short extraneous outgrowths and a clublike expansion arising from its single process. Gasserian ganglion. Female, 66 years old. Cajal's technic. $\times 1000$.
- FIG. 4. Truncated multipolar neuron. Celiac ganglion. Female, 42 years old. Cajal's technic. $\times 1000$.
- FIG. 5. Truncated multipolar neuron with moderate hypertrophy of processes. Third lumbar sympathetic ganglion. Male, 59 years old. Bodian's technic. $\times 1000$.

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PLATE 46

- FIG. 6. Degenerate unipolar neuron possessing three thick intracapsular processes. Gasserian ganglion. Male, 65 years old. Cajal's technic. $\times 800$.
- FIG. 7. Multipolar neuron with similar number of processes as cell in Figure 6. Celiac ganglion. Female, 42 years old. Cajal's technic. $\times 800$.
- FIG. 8. Multipolar neuron with three processes. Third lumbar sympathetic ganglion. Male, 34 years old. Bodian's technic. $\times 800$.
- FIG. 9. Degenerate sensory neuron with many short accessory processes arising from cell soma. Gasserian ganglion. Female, 66 years old. Cajal's technic. $\times 800$.
- FIG. 10. Multipolar cell with processes arising from cell soma. Celiac ganglion. Female, 42 years old. Cajal's technic. $\times 800$.
- FIG. 11. Multipolar neuron with short dendritic processes. Note that all processes do not penetrate the investing capsule. Third lumbar sympathetic ganglion. Male, 59 years old. Bodian's technic. $\times 800$.
- FIG. 12. Degenerate sensory neuron with supernumerary processes arising from entire circumference of cell. Note variation in thickness and terminal expansions of these radicles. Gasserian ganglion. Male, 65 years old. Cajal's technic. $\times 800$.
- FIG. 13. Multipolar neuron with branching processes. Third lumbar sympathetic ganglion. Male, 77 years old. Cajal's technic. $\times 800$.
- FIG. 14. Multipolar neuron with short branching processes. Note similarity to degenerate neuron in Figure 12. Third lumbar sympathetic ganglion. Male, 59 years old. Bodian's technic. $\times 800$.

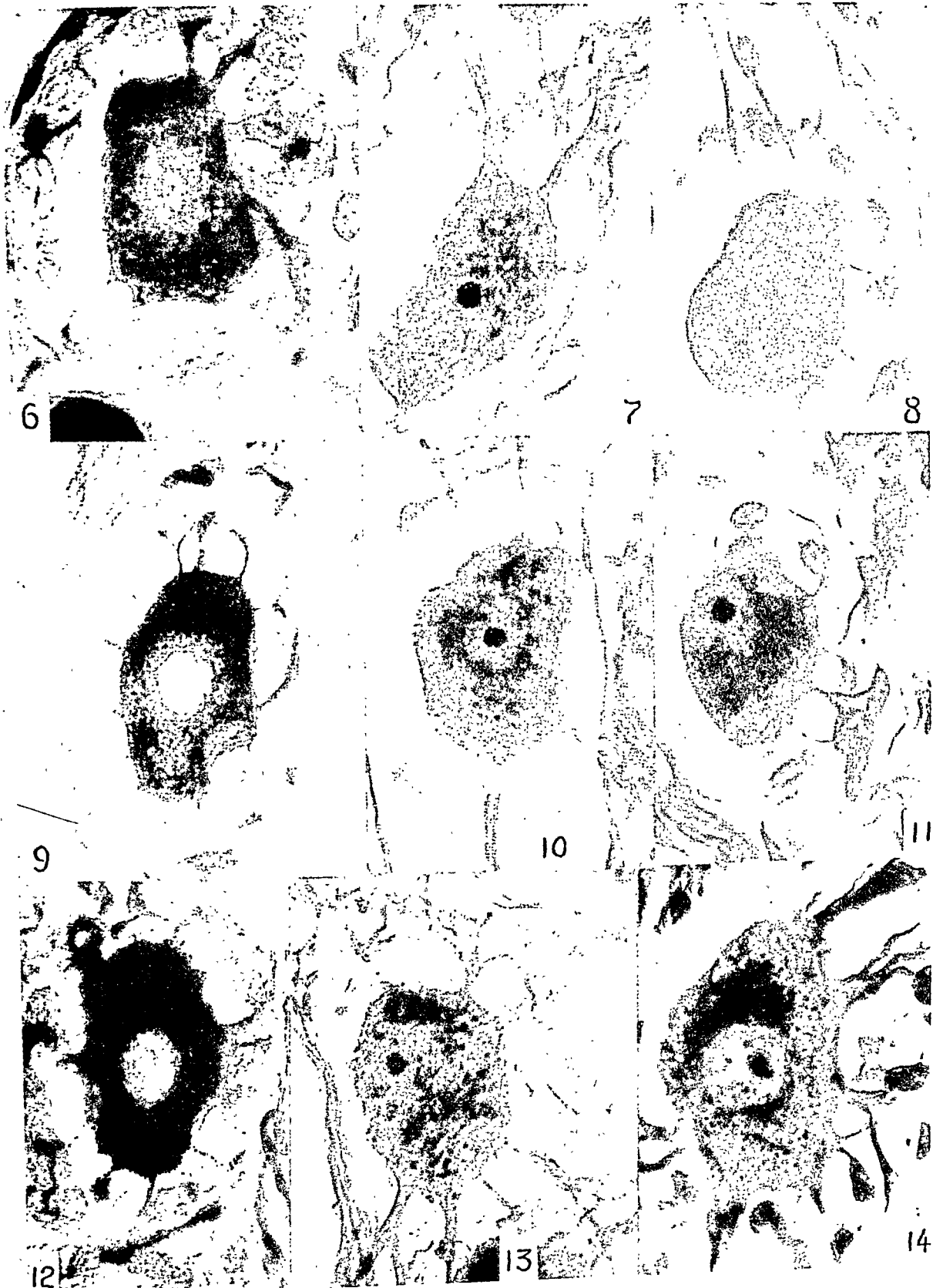


Truex

Degenerate Versus Multipolar Neurons

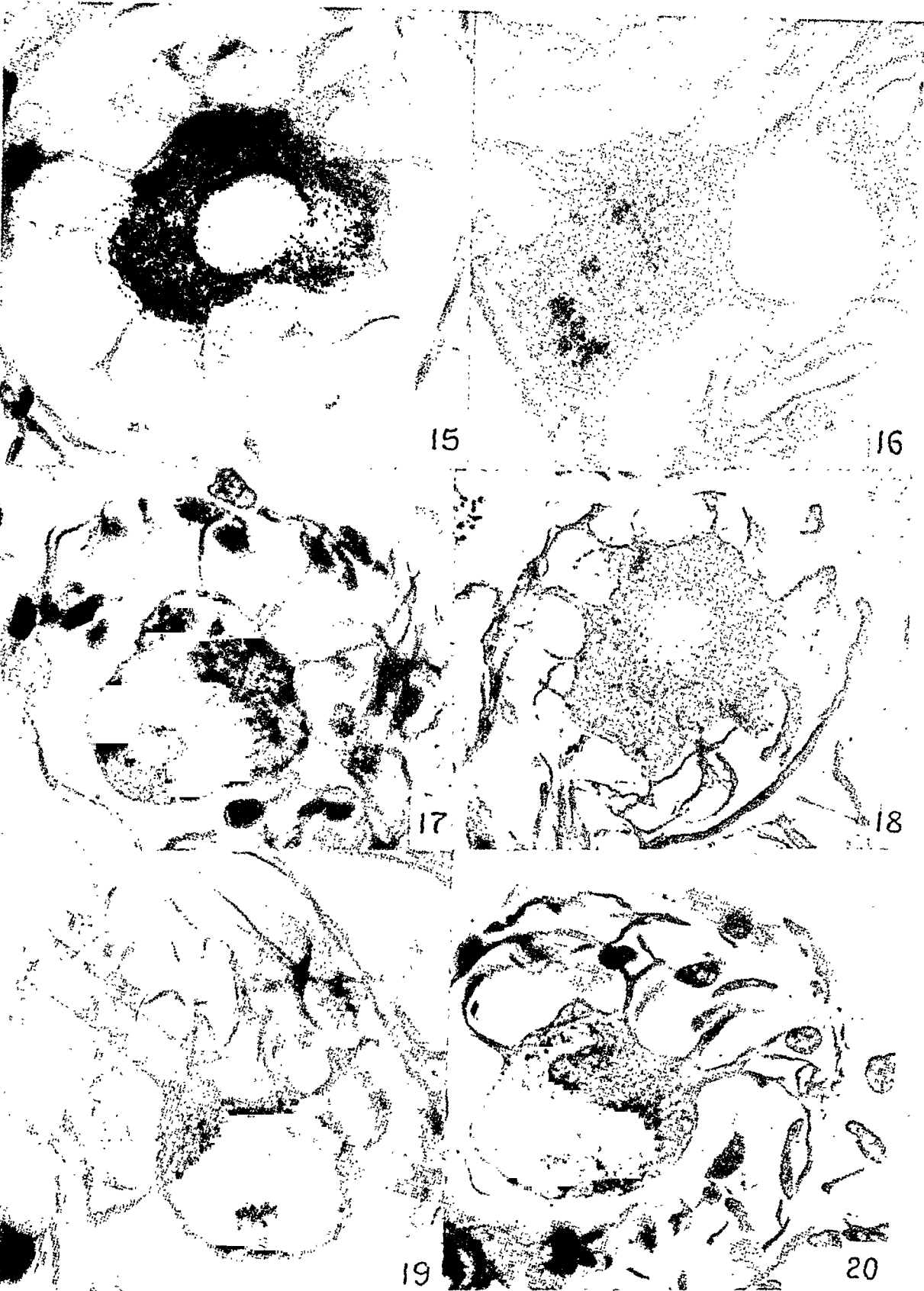
PLATE 47

- FIG. 15. Degenerate unipolar neuron demonstrating short branching processes. Gasserian ganglion. Male, 65 years old. Cajal's technic. $\times 800$.
- FIG. 16. Multipolar neuron with process formation similar to the degenerate cell in Figure 15. Female, 42 years old. Cajal's technic. $\times 800$.
- FIG. 17. Multipolar neuron with short intracapsular processes. Third lumbar sympathetic ganglion. Male, 59 years old. Bodian's technic. $\times 800$.
- FIG. 18. Degenerate unipolar neuron with extreme branching of processes. Note the wealth of primary, secondary and tertiary rami on this cell. Gasserian ganglion. Female, 66 years old. Cajal's technic. $\times 800$.
- FIG. 19. Multipolar neuron with process formation similar to the degenerate sensory cell shown in Figure 18. Third lumbar sympathetic ganglion. Male, 77 years old. Cajal's technic. $\times 800$.
- FIG. 20. Multipolar neuron. Commonly designated as "comet-cell" of sympathetic nervous system. Note intracapsular branching of processes. Celiac ganglion. Male, 55 years old. Bodian's technic. $\times 800$.



Truex

• Degenerate Versus Multipolar Neurons



similar variations, consisting of two rises, one at 10 a.m. to 12 noon, and the other at 3 to 4 p.m. There were also sudden variations of about 2500 leukocytes at short intervals.

In these reports, no mention is made of the number of 1 sq. mm. unit areas which were examined in determining the total leukocyte counts of each rabbit. Sabin and co-workers¹ did not state the number of leukocytes which they studied in making the differential examinations of their rabbit. Bushnell and Bangs² counted and classified approximately 200 leukocytes in making their differential determinations.

Despite the scarcity of reports of daily leukocyte variations of individual rabbits, a voluminous literature has accumulated concerning other aspects of leukocyte variations of rabbits. This literature has been summarized by Cheng³ and Garrey and Bryan.⁴ An analysis of errors in leukocyte counting has been presented by Bryan, Chastain and Garrey.⁵ Anyone working with variations in leukocyte counts is impressed with the numerous possible sources of error in the determinations, involving all procedures from selection of subjects to the actual counting of the leukocytes.

A large number of cells should be counted in making total and differential leukocyte determinations if significant results are to be obtained. The importance of counting enough cells in making total leukocyte counts is evident from studies of leukocyte variations in normal human subjects by Sabin and co-workers¹ and Ponder, Saslow and Schweizer.⁶ Sabin *et al.*, counting the cells on "the same side of a counting chamber" in five of six total leukocyte determinations, described hourly rhythmical leukocyte variations. Ponder and co-workers obtained similar apparent hourly rhythms when the cells on "one side of one counting chamber" were counted, but noted that these rhythms tended to disappear and much less fluctuation in counts occurred when the cells on "both sides of 2 counting chambers" were counted. It seems advisable to record the number of cells enumerated in making total leukocyte counts in terms of a standard, such as "unit areas of one square millimeter each," recognizing that one side of a certified counting chamber contains nine such unit areas.⁶

After every attempt has been made to eliminate possible sources of error in the determinations, irregular variations may

I. HOURLY LEUKOCYTE VARIATIONS OF NORMAL RABBITS

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Few studies have been reported of the variations of individual rabbits whose leukocytes have been counted at short intervals during the day. The purpose of this report is to present results of total and differential leukocyte examinations of 14 normal rabbits, studied at hourly intervals.

In the report by Sabin, Cunningham, Doan and Kindwall¹ of "the normal rhythm of the white blood cells" in human subjects, brief mention is made that experiments on the blood of rabbits, similar to the reported experiments on humans, showed "the same general rhythms." They published one chart of one rabbit, studied at consecutive 30 minute intervals from 11 a.m. to 1:15 p.m. This chart shows considerable variation in total leukocyte counts and numbers of polymorphonuclear neutrophilic leukocytes (amphophils) and lymphocytes. Bushnell and Bangs² tabulated the total and differential leukocyte counts of one rabbit, examined at consecutive 45 to 60 minute intervals during the day on five occasions over a period of 43 days. On each day no determinations were made during a 2-hour interval around noon. Under these conditions, considerable daily variation occurred in the leukocyte counts of this rabbit. Cheng³ presented the total leukocyte counts of two rabbits, studied at consecutive 15 to 30 minute intervals. One rabbit was examined during the afternoon of one day and again during the morning of the following day. The other rabbit was studied during the afternoon of one day and again, 2 days later, from late morning through the afternoon. Cheng stated that the total counts of both rabbits tended to show

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guaranteed to contain the following ingredients: wheat germ, soy bean oil meal, cut alfalfa, crushed oats, crushed barley, wheat middlings (standard), corn germ meal, crushed corn, molasses, calcium carbonate (limestone) 0.5 per cent, and iodized salt 0.5 per cent. No changes were made in feeding on the days of counting.

Leukocyte counts were made during the same general period of the day, beginning at approximately 9 to 9:45 a.m., with hourly determinations through 3:45 to 4 p.m. All total and differential examinations were made by one individual. Special consideration was given to the handling of the rabbits and all other procedures dealing with the collection and examination of blood samples to ensure the utmost accuracy of the results.

Blood was obtained from the lateral marginal vein of the left ear, punctured by a Hagedorn needle. Freely flowing drops of blood were considered essential. The same certified pipette was used for an animal throughout a day of counting. The diluting fluid was 1 per cent acetic acid, freshly prepared and filtered. Pipettes were shaken by hand for 3 minutes and discharged into duplicate counting chambers after the first three or four drops had been discarded. Certified Zeiss chambers with the Neubauer ruling and polished glass shoulders were used, and coverglasses were carefully adjusted so that proper depth of the counting chambers was obtained. Approximately 10 minutes was allowed for settling of the leukocytes.

The cells in the four large corner squares of both sides of two chambers were counted, so that 800 cells were actually enumerated when the total number of leukocytes was 10,000 per cu. mm. Thus in each case sixteen unit areas of 1 sq. mm. each were examined.

At least three blood smears were prepared for each hourly count. Smears were made on slides which had been freshly cleaned with acid alcohol and polished with a clean dry towel. Films were stained with Wright's stain and studied with the oil immersion objective, at a magnification of $\times 970$. In each case, 300 leukocytes were counted, essentially the same areas being examined on each slide.

Leukocytes were classified into five general groups: polymorphonuclear neutrophils (amphophils), lymphocytes, large

still occur in leukocyte counts of rabbits made at short intervals during the day.^{4,7} Observations of human leukocyte variations indicate that fairly frequently the total leukocyte count increases moderately in the afternoon, so that the average of the afternoon counts may be higher than the average of the morning counts.^{1,6,8} In many instances this afternoon increase in human subjects is apparently due mainly to an absolute increase in the number of polymorphonuclear neutrophilic leukocytes.^{1,8,9}

The following observations of hourly leukocyte variations of 14 normal rabbits are presented. These studies were made in connection with experiments on leukocytosis to be reported subsequently.

PROCEDURE

Fourteen adult New Zealand white and mixed rabbits, weighing approximately 2.5 to 3.2 Kg. each, were studied during the period from May 24, 1939 to January 23, 1940. Each animal was kept in the same individual cage throughout this period and for several months prior to the day of counting. During this time the rabbits were removed from their cages at least once daily and appeared adjusted to handling. The temperature of the cages and counting rooms was maintained at approximately 20 to 25° C. One animal (B) was counted for two series of observations. No differences were noted between the counts of males and females, and both sexes were used. Rabbits with ear canker, snuffles, or any other abnormality, including abnormal eating or activity, were discarded. As the incidence of coccidia infestation appears worthy of consideration in reports dealing with leukocyte variations of the rabbit,¹⁰ mention should be made that the incidence of coccidiosis has been low in autopsied animals of similar breeds kept under identical conditions.

Food was placed in each rabbit cage once daily at approximately the same time (9 to 11 a.m.). The animals ate slowly over a period of 3 to 4 hours. The diet consisted of a complete ration chow, carrots and water. The rabbit chow was guaranteed by the manufacturer to have the following analysis: crude protein not less than 13.5 per cent, crude fat not less than 2.5 per cent, carbohydrate in crude fiber not less than 16 per cent, and carbohydrate in nitrogen-free extract not less than 47 per cent. It was

TABLE I—(Continued)

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leukocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
C	9:30 a.m.*						
	10:00 a.m.	11800	34	56	2	1	8
	11:00 a.m.	8600	27	64	1	2	5
	12:00 noon	7700	36	54	2	1	6
	1:00 p.m.	8600	45	46	1	1	8
	2:00 p.m.	11500	41	43	1	0	15
	3:00 p.m.	7200	39	47	1	1	12
D	9:40 a.m.*						
	9:00 a.m.	7300	11	86	1	0	2
	10:00 a.m.	6900	23	70	1	1	5
	11:00 a.m.	7500	22	71	2	1	4
	12:00 noon	10400	33	64	1	0	2
	1:00 p.m.	7700	39	55	2	0	4
	2:00 p.m.	8400	38	56	2	1	3
E	10:00 a.m.*						
	9:55 a.m.	5500	41	55	1	1	2
	10:55 a.m.	5600	35	60	1	1	3
	11:55 a.m.	6300	31	62	3	1	4
	12:55 p.m.	11100	35	62	0	2	2
	1:55 p.m.	4600	34	60	1	2	3
	2:55 p.m.	6300	49	46	1	0	4
F	3:55 p.m.	8200	55	40	0	0	5
	9:30 a.m.*						
	10:05 a.m.	10600	23	72	1	1	2
	11:05 a.m.	7900	23	75	1	0	1
	12:05 p.m.	9700	21	72	1	2	4
	1:05 p.m.	8900	17	80	1	0	2
	2:05 p.m.	10200	24	72	1	1	2
G	3:05 p.m.	11000	29	64	1	0	6
	4:05 p.m.	10800	37	56	1	1	4
	10:30 a.m.*						
	9:45 a.m.	11300	24	70	4	0	2
	10:45 a.m.	10200	36	58	3	0	2
	11:45 a.m.	9600	33	56	5	0	6
	12:45 p.m.	8900	31	60	3	0	6
H	1:45 p.m.	10600	37	55	3	1	5
	2:45 p.m.	8300	32	63	3	0	3
	3:45 p.m.	8900	29	63	2	0	6
	10:00 a.m.*						
	9:45 a.m.	8200	41	53	2	0	4
	10:45 a.m.	9000	39	56	1	1	4
	11:45 a.m.	9800	39	55	1	0	5
	12:45 p.m.	8300	39	53	1	1	6
	1:45 p.m.	7900	45	49	0	1	5
	2:45 p.m.	9200	50	42	0	1	7
	3:45 p.m.	8300	47	50	0	0	3

* Indicates time of feeding.

mononuclear cells (monocytes), eosinophils, and basophils. In the present study, neutrophils (amphophils) were not subdivided into classes according to the appearance of the nuclear mass. The number of unclassified cells was less than 0.5 per cent in each case. Confusion was only occasionally encountered between lymphocytes and large mononuclear cells.

Total leukocyte counts were recorded to the nearest hundred. Differential percentages were adjusted to the nearest integer. Averages of the hourly total and absolute leukocyte counts and differential percentages of all rabbits were calculated by averaging counts made at seven consecutive hourly periods (from 9:45 to 10:05 a.m. through 3:45 to 4:05 p.m.). Averages of all counts of the day for each rabbit were also obtained. Morning and afternoon averages for individual rabbits were obtained by averaging all determinations during each respective period. In Table VI, differences between morning and afternoon averages are expressed as percentages of the morning average in each case.

TABLE I
Differential Leukocyte Examinations of 14 Normal Rabbits

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leukocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
A	9:15 a.m.*						
	9:25 a.m.	7000	22	71	2	0	6
	10:25 a.m.	8800	21	71	1	1	6
	11:25 a.m.	7700	34	60	2	1	3
	12:25 p.m.	6600	32	60	3	1	5
	1:25 p.m.	11900	34	61	1	0	4
	2:25 p.m.	12200	33	65	1	1	1
	3:25 p.m.	14900	38	57	1	1	3
B	9:15 a.m.*						
	10:00 a.m.	7000	13	81	2	0	3
	11:00 a.m.	5900	14	80	2	1	3
	12:00 noon	5100	13	82	2	0	3
	1:00 p.m.	6500	25	65	2	3	5
	2:00 p.m.	7000	20	74	1	0	4
	3:00 p.m.	7900	49	45	2	1	4
B	11:00 a.m.*						
	9:45 a.m.	6000	10	86	2	0	2
	10:45 a.m.	5600	16	81	1	0	2
	11:45 a.m.	5800	23	73	0	0	3
	12:45 p.m.	9500	46	51	1	0	2
	1:45 p.m.	7100	33	62	1	1	3
	2:45 p.m.	6700	56	39	0	0	4
	3:45 p.m.	6700	52	43	1	0	4

* Indicates time of feeding.

RESULTS

Results are presented in tabular form. Table I shows for each experiment the times of counts, the times that food was placed in the rabbit cages, total numbers of leukocytes per cu. mm. and differential percentages of neutrophils (amphophils), lymphocytes, large mononuclear cells, eosinophils, and basophils. As may be seen, there is considerable daily variation in different experiments and the time of placing food in the cages seems to bear no direct relationship to these variations. Variations in averages of all counts of the day of each rabbit, presented in Table II, likewise are of considerable magnitude. Repeated leuko-

TABLE II

Averages of Total Leukocyte Counts, Differential Percentages and Absolute Numbers of Leukocytes for All Counts of the Day of 14 Normal Rabbits

Rabbit letter	Average total leukocytes per cu. mm.	Average differential percentages and absolute numbers of leukocytes per cu. mm.			
		Neutrophils (amphophils)		Lymphocytes	
		Per cent	Absolute number	Per cent	Absolute number
A	9871	30	3122	63	6211
B	6566	22	1549	71	4579
B	6771	33	2397	62	4095
C	9233	37	3416	52	4759
D	8033	27	2277	67	5338
E	6800	40	2744	55	3730
F	9871	25	2491	70	6870
G	9685	31	3067	61	5886
H	8671	43	3712	51	4437
I	10642	14	1485	79	8496
J	10283	30	3090	61	6287
K	12600	30	3786	63	8047
L	15014	42	6389	52	7873
M	11285	21	2291	75	8524
N	6414	37	2461	53	3362
Average	9449	31	2952	62	5900

cyte examinations of 3 animals at approximately the same time on different days, prior or subsequent to the day of hourly counting, tend to show that, in general, each rabbit seems to have its own characteristic leukocyte picture and variations appear to be decreased under such conditions (Table III). Repeated leukocyte examinations of the other rabbits showed similar results.

Averages of the hourly total and absolute leukocyte counts and differential percentages of all 14 rabbits (Table IV) fail to reflect

TABLE I—(Continued)

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leucocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
I	10:00 a.m.*						
	9:45 a.m.	12200	11	87	1	0	1
	10:45 a.m.	10600	11	84	2	0	3
	11:45 a.m.	10100	14	82	2	0	3
	12:45 p.m.	11600	12	76	3	1	8
	1:45 p.m.	9000	18	72	2	1	7
	2:45 p.m.	10500	18	76	1	2	4
	3:45 p.m.	10500	15	80	1	0	3
J	9:30 a.m.*						
	10:45 a.m.	9300	21	69	2	0	7
	11:45 a.m.	9800	27	65	2	2	5
	12:45 p.m.	11700	28	63	2	1	5
	1:45 p.m.	11600	33	59	1	2	6
	2:45 p.m.	10000	34	57	2	1	7
	3:45 p.m.	9300	37	54	3	0	6
K	11:00 a.m.*						
	9:45 a.m.	12500	21	75	2	1	1
	10:45 a.m.	12100	21	71	3	0	5
	11:45 a.m.	15700	25	68	1	0	5
	12:45 p.m.	14200	34	60	2	0	4
	1:45 p.m.	10200	43	53	1	1	2
	2:45 p.m.	12600	40	53	1	1	5
	3:45 p.m.	10900	29	65	1	0	5
L	11:00 a.m.*						
	9:55 a.m.	15800	38	56	1	1	4
	10:55 a.m.	16200	39	57	3	0	1
	11:55 a.m.	14300	45	45	3	1	6
	12:55 p.m.	16200	48	47	2	1	3
	1:55 p.m.	13700	40	57	1	0	1
	2:55 p.m.	14300	44	52	1	0	3
	3:55 p.m.	14600	44	53	1	0	2
M	10:30 a.m.*						
	9:45 a.m.	9100	5	90	0	0	4
	10:45 a.m.	15000	14	83	1	0	2
	11:45 a.m.	13800	15	81	0	0	4
	12:45 p.m.	9900	30	66	1	0	3
	1:45 p.m.	9800	36	58	1	1	4
	2:45 p.m.	10500	25	70	1	1	4
	3:45 p.m.	10900	21	76	0	0	2
N	10:30 a.m.*						
	9:55 a.m.	5200	13	70	3	3	11
	10:55 a.m.	5600	27	65	3	0	5
	11:55 a.m.	6600	38	53	1	1	7
	12:55 p.m.	5700	44	47	1	3	4
	1:55 p.m.	6700	51	43	0	1	4
	2:55 p.m.	6400	38	50	1	2	9
	3:55 p.m.	8700	48	46	1	1	4

* Indicates time of feeding.

the extent of the variations evident from the previous tables dealing with individual animals. When only average determinations are considered, in general the total leukocyte values are fairly constant, the absolute number of lymphocytes slightly decreases and the absolute number of neutrophils (amphophils) slightly increases during the day. Consequently, the differential percentages of lymphocytes and neutrophils (amphophils) slightly decrease and slightly increase, respectively. Percentages and absolute numbers of large mononuclear cells, eosinophils and basophils fluctuate but cannot be considered to exhibit significant trends because these cells comprise only a small fraction of the circulating leukocyte population.

TABLE V
*Increase or Decrease of Afternoon Average Leukocyte Counts, with Reference to Morning Average Leukocyte Counts, in 14 Normal Rabbits**

Rabbit letter	Total leukocytes	Neutrophils (amphophils)	Lymphocytes
A	+3567	+1959	+1664
B	+175	+1022	-910
B	+1700	+2544	-958
C	-1450	+374	-1945
D	+1600	+1853	-280
E	+1750	+1205	+525
F	+870	+510	+129
G	-1191	-205	-900
H	-575	+258	-854
I	-566	+312	-1347
J	+1100	+1187	-159
K	-1458	+1324	-2625
L	-733	+241	-523
M	-2358	+1311	-3643
N	+1075	+1568	-403

* A positive sign indicates that the afternoon average was greater than the morning average by the total difference indicated. A negative sign indicates that the afternoon average was less than the morning average by the total difference indicated.

The increase or decrease of the average of the afternoon total leukocyte counts of individual rabbits, with reference to the average of the morning determinations in each case, is shown in Table V. It is evident that an apparent increase in the average of the afternoon total leukocyte counts was a fairly frequent but not constant finding. These increases or decreases are expressed as percentages of the respective morning averages in each case in Table VI. In this table the afternoon leukocyte fluctuations noted in studies of normal human leukocyte variations by Sabin and co-workers,¹ Shaw,⁸ and Ponder, Saslow and Schweizer,⁶ and ex-

TABLE III

Repeated Leukocyte Examinations of 3 Normal Rabbits at Approximately the Same Time of Day on Different Days

Rabbit letter	Time	Total leukocytes per cu. mm.	Neutrophils (amphophils)		Lymphocytes	
			Per cent	Absolute number	Per cent	Absolute number
B	10:00 a.m.	9500	11	1045	83	7885
	10:30 a.m.	7200	11	792	84	6048
	10:20 a.m.	7000	5	350	88	6160
	10:00 a.m.	7000	13	910	81	5670
	9:45 a.m.	6000	10	600	86	5160
	9:40 a.m.	6300	8	504	87	5481
I	9:45 a.m.	12200	11	1342	87	10614
	9:05 a.m.	11300	7	791	87	9831
	9:30 a.m.	14400	7	1008	86	12384
	9:42 a.m.	14700	8	1176	87	12789
	9:45 a.m.	12700	8	1016	89	11303
	9:55 a.m.	5200	13	676	70	3640
N	8:55 a.m.	7100	22	1562	66	4686
	8:55 a.m.	5700	9	513	81	4617
	9:25 a.m.	6000	13	780	76	4650
	9:35 a.m.	5800	22	1276	63	3654

TABLE IV

Averages of Hourly Leukocyte Counts of 14 Normal Rabbits

Time of count	Number of determinations averaged	Average total leukocytes per cu. mm.	Average differential percentages and absolute numbers of leukocytes per cu. mm.			
			Neutrophils (amphophils)		Lymphocytes	
			Per cent	Absolute number	Per cent	Absolute number
9:45 a.m. to 10:05 a.m.	12	9600	23	2282	71	6751
10:45 a.m. to 11:05 a.m.	14	9221	24	2303	69	6397
11:45 a.m. to 12:05 p.m.	14	9621	28	2727	65	6237
12:45 p.m. to 1:05 p.m.	14	9914	33	3358	59	5895
1:45 p.m. to 2:05 p.m.	14	9164	35	3242	58	5295
2:45 p.m. to 3:05 p.m.	13	9300	38	3511	54	5148
3:45 p.m. to 4:05 p.m.	11	9800	38	3594	56	5681

termine the general normal leukocyte trends of each animal and then maintain the same period of the day for counting at hourly intervals under experimental conditions, as well as to establish similar control of other possible sources of error in the determinations.

SUMMARY

Hourly total and differential leukocyte studies of 14 normal rabbits are presented.

Individual examinations show considerable variation; average counts exhibit tendencies for the total numbers of leukocytes to vary little, and for the percentages and absolute numbers of lymphocytes and neutrophils (amphophils) to decrease and increase slightly, respectively, during the day.

An apparent afternoon increase in the number of neutrophils (amphophils) is a more frequent finding than an apparent afternoon increase in the total leukocyte count.

In studying the hourly leukocyte picture of rabbits under different experimental conditions, it would seem advisable to determine general normal leukocyte trends for each animal and then establish the same periods of the day for making counts under experimental conditions.

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pressed as percentage increases or decreases (with reference to respective morning averages) by Ponder and co-workers,⁶ are compared with similarly expressed percentage increases or decreases in individual rabbits in the present study. Although comparison of human and rabbit experiments may be unjustifiable, it should be emphasized that expression of differences between two variables in terms of percentage of one of these variables may introduce confusion in interpretation of findings. Since biometric analyses seem unjustified in view of the small number of variants in the present study, actual numerical increases or decreases of the individual afternoon averages are listed in Table V.

Further examination of Table V shows that, in the 14 rabbits studied, an apparent afternoon increase in the number of neutrophils (amphophils) appeared to be more frequent than an afternoon rise in the total leukocyte count. An apparent afternoon decrease in the number of lymphocytes seemed to be a fairly frequent, although inconstant, finding. Variations in numbers of blood lymphocytes may, to a large extent, reflect similar changes in lymph flow.⁴

From a practical standpoint, these results seem to indicate that when the hourly leukocyte picture of the rabbit is studied under different experimental conditions, it would seem advisable to de-

TABLE VI
Increase or Decrease of Afternoon Average Total Leukocyte Counts, Expressed as Percentages of Respective Morning Average Leukocyte Counts, in Normal Human Subjects and in 14 Normal Rabbits

Subject	Percentage relationship as found by different authors			
	Normal human subjects			14 normal rabbits
	Ponder <i>et al.</i> ⁶	Shaw ⁸	Sabin <i>et al.</i> ¹	Reifenstein <i>et al.</i>
1	3	26	17	45
2	1	15	15	-13
3	12	-13	14	22
4	4	28	9	30
5	10	30	6	9
6	-24	13	24	-11
7	57	35	..	-6
8	13	20	..	-5
9	..	3	..	11
10	..	15	..	-11
11	-5
12	-18
13	18
14a*	3
14b*	29

* 14a and 14b represent the same rabbit (B) counted on two occasions.

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supernatant fluid fraction retained. In a series of 13 experiments with 9 rabbits, each rabbit received the same amount (5 cc.) of such supernatant fluid by intravenous injection. Total and differential leukocyte studies of these rabbits were made at hourly intervals after the intravenous injection. These observations were compared with similar leukocyte determinations following intravenous injections of similar amounts of sterile saline solutions, of the same chloride concentrations as the supernatant fluid fractions, using the same experimental animals on five occasions.

PROCEDURE

The method used for the production of exudates was similar to those of de Haan;¹ Mudd, Lucké, McCutcheon and Strumia;² Ponder and Macleod;³ and Coman.⁴ A saline solution was prepared by dissolving 9 gm. of sodium chloride, C. P. (Merck), in water doubly distilled from glass, to a total volume of 1 L. The solution was autoclaved at 15 pounds pressure for 30 minutes. The P_H of this solution before and after sterilization was approximately 6.2, as determined colorimetrically and by the quinhydrone electrode. Approximately 300 cc. of this sterile solution was injected at room temperature, without anesthesia and by gravity flow, into the peritoneal cavity of a rabbit. After 5¾ to 12 hours, the fluid exudate was withdrawn under sterile conditions without anesthesia, using a large bore needle, rubber tubing and a glass suction bottle. The periods of exudation occurred at various times of the day and night. Repeated exudates were produced in some animals, but the shortest period of time between intraperitoneal injections in the same rabbit was 10 days. No essential differences were noted between the first and subsequent exudates but occasionally, after repeated injections, fibrous adhesions in the peritoneal cavity seemed to hinder removal.

Repeated bacteriological examinations of sterilized saline, whole exudates and supernatant fluids, using direct smears, blood plates, infusion agar and corn syrup agar, yielded negative results.

The amount of exudate obtained varied up to 154 cc., apparently depending on the duration of exudation and the individual rabbit. Several times when no fluid was obtained, none could be found upon immediate postmortem examination of the animal. In these cases there were no indications that the fluid had been

STUDIES ON LEUKOCYTOSIS *

II. NEUTROPHILIC LEUKOCYTOSIS FOLLOWING INTRAVENOUS INJECTION OF SUPERNATANT FLUID FROM A STERILE EXUDATE (RABBIT)

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It is generally postulated that the polymorphonuclear neutrophilic leukocytosis associated with acute inflammation is due to the presence in the inflamed area of some substance which enters the blood stream and effects a transient increase in the number of leukocytes (polymorphonuclear neutrophils) in the circulating blood. This increase apparently occurs rather rapidly during the course of several hours. Such a neutrophilic increase may be accompanied by a normal total leukocyte count (*relative neutrophilic leukocytosis*), or may be associated with a variable increase in the total leukocyte count hereafter referred to as an *absolute neutrophilic leukocytosis*.

If such a substance exists, the intravenous injection into one animal of a relatively small amount of sterile exudate, of less than 24 hours' duration in an homologous animal, should be followed by a transient increase in the number of polymorphonuclear neutrophilic leukocytes in the circulating blood of the injected animal. Such a neutrophilic rise should occur a relatively short time after injection. Perhaps in some instances an absolute neutrophilic leukocytosis might occur.

In the present study of experimental leukocytosis, sterile procedures were employed throughout all experiments. Sterile 0.9 per cent saline solution was injected intraperitoneally into rabbits to produce peritoneal exudates. In each experiment this exudate was withdrawn 5¾ to 12 hours later, centrifugalized and its

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TABLE I
Results of All Experiments on Leukocytosis (9 Rabbits)

Rabbit letter	Experiment number	Experimental procedure	Hourly total leukocyte counts per cu. mm.							Hourly neutrophil (ampho-phil) percentages							Hourly lymphocyte percentages						
			1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
B	39.10	No injection	7000	5900	5100	6500	7000	7900	13	14	13	25	20	49	..	81	80	82	65	74	45	..
B	39.39	No injection	6000	5600	5800	9500	7100	6700	6700	10	16	23	46	33	56	52	86	81	73	51	62	39	43
B	39.48	Super. fluid	7100	6800	12600	10300	8300	8100	8800	18	38	80	71	71	65	65	74	56	17	25	24	30	26
B	40.22	Super. fluid	6300	5900	9300	8400	7500	8500	6900	8	30	78	67	75	65	71	87	65	19	26	23	30	26
B	39.2	0.90% NaCl	6000	5900	7200	6400	7500	6200	6900	15	13	34	48	46	40	38	77	81	56	35	43	46	53
F	39.33	No injection	10600	7900	9700	8900	10200	11000	10800	23	23	21	17	24	29	37	72	75	72	80	72	64	56
F	39.47	Super. fluid	7500	5100	12900	11700	9700	9700	10600	20	14	44	63	50	51	47	71	79	44	29	42	39	46
F	40.26	Super. fluid	6700	8200	10500	10000	9300	9300	8200	17	20	54	40	44	35	44	74	69	40	51	49	60	49
F	40.30	0.78% NaCl	8000	8800	10000	7400	10100	8400	7000	21	28	23	30	30	26	32	75	70	76	68	67	72	66
H	39.40	No injection	8200	9000	9800	8300	7900	9200	8300	41	39	39	39	45	50	47	53	56	55	53	49	42	50
H	39.49	Super. fluid	8200	14500	29900	32000	26100	25300	24800	9	15	38	42	42	38	31	88	84	60	54	53	59	65
I	39.43	No injection	12200	10600	10100	11600	9000	10500	10500	11	11	14	12	18	18	15	87	84	82	76	72	76	80
I	40.1	Super. fluid	14400	14400	11200	11900	13500	10000	13200	7	15	47	34	28	32	19	86	73	50	61	66	57	77
I	40.24	Super. fluid	14700	13100	15600	15700	15400	14500	18300	8	10	28	26	30	15	21	87	87	68	72	65	81	76
I	40.29	0.78% NaCl	12700	10300	10700	14300	16500	14900	15700	8	6	17	19	16	11	18	89	91	79	79	83	88	79
J	39.44	No injection	9300	9800	11700	11600	10000	9300	..	21	27	28	33	34	37	..	69	65	65	59	57	54
J	40.2	Super. fluid	12700	13300	14000	12500	12700	13900	12100	10	24	55	49	49	51	43	83	65	39	46	45	43	46
K	40.4	No injection	12500	12100	15700	14200	10200	12600	10900	21	21	25	34	43	40	29	75	71	68	60	53	53	65
K	40.8	Super. fluid	11200	8600	10900	11200	12100	11700	13400	13	32	55	54	52	45	44	76	64	43	45	41	49	45
K	40.23	Super. fluid	11500	9200	6400	10200	10700	10100	15400	20	37	59	68	51	61	45	77	59	36	30	44	34	49
L	40.5	No injection	15800	16200	14300	16200	13700	14300	14600	38	39	45	48	40	44	44	56	57	45	47	57	52	53
L	40.9	Super. fluid	12400	8200	12600	16800	12800	10500	12600	26	33	57	67	58	69	72	68	64	41	29	38	25	24
M	40.6	No injection	9100	15000	13800	9900	9800	10500	10900	5	14	15	30	36	25	21	90	83	81	66	58	70	76
M	40.10	Super. fluid	12900	11300	13000	14200	13500	13300	11800	13	37	39	45	44	43	36	83	59	57	52	50	54	60
M	40.31	0.85% NaCl	12200	13100	12200	14100	13000	13200	14200	10	7	9	20	19	16	18	86	90	89	76	79	83	80
N	40.7	No injection	5200	5600	6600	5700	6700	6400	8700	13	27	38	44	51	38	48	70	65	53	47	43	50	46
N	40.11	Super. fluid	7100	9900	10900	9700	8800	9900	8000	22	46	53	47	47	42	37	66	43	35	41	44	47	52
N	40.32	0.85% NaCl	5800	5600	6400	7900	8500	11200	11900	22	19	17	30	39	35	42	63	76	75	67	55	56	53

injected into the urinary bladder or gastro-intestinal tract. Of 75 instances in which an attempt was made to withdraw fluid, no appreciable exudate could be obtained in 15.

The exudate was colorless, opalescent, cloudy or grossly purulent and sometimes contained small flecks of fibrinous material. Occasionally the fluid clotted immediately or shortly after removal. The specific gravity ranged from 1.012 to 1.022. The total leukocytes per cu. mm. varied from several hundred to 33,000, of which approximately 80 to 95 per cent were polymorphonuclear neutrophils (amphophils) and the remainder were lymphocytes and monocytes.

After samples were taken for leukocyte counts and bacteriological examinations, the sterile exudate was immediately centrifugalized for 15 minutes at a speed of 3125 r. p. m. Fibrin clots adherent to the centrifuge tube were discarded. The supernatant fluid was carefully decanted or pipetted off. Five cc. of this fluid was immediately injected intravenously into the rabbit to be studied, using the lateral marginal vein of the previously shaved right ear. In performing the injection, care was taken to avoid infiltration of fluid into the ear, thrombosis or excessive trauma, and the vein was examined for patency and freedom of venous flow following the injection.

Nine rabbits were used in the present experiments. Each rabbit received one intravenous injection of 5 cc. of supernatant fluid and 4 of the 9 (animals B, F, I and K) received a second such injection after an interval of 58 to 106 days.

Using Whitehorn's⁵ method, determinations were made of the chloride concentrations of the supernatant fractions of the centrifugalized exudates. They varied between 0.90 and 0.78 gm. of sodium chloride per 100 cc. of supernatant fluid. As a control procedure, sterile sodium chloride solutions, containing 0.90 to 0.78 gm. of sodium chloride per cent, were prepared in the described manner and injected intravenously into 5 of the 9 rabbits in an equivalent amount (5 cc.).

The various procedures involved in leukocyte determinations, such as feeding, housing, selection of animals, obtaining of blood samples and actual counting, have been described previously.⁶ Special consideration was given to the exact duplication of these methods and to all other procedures to ensure the utmost accuracy

This fact is more evident in Chart 1, in which the leukocyte curves of rabbit B without injection (experiment No. 39.10), following supernatant fluid injection (experiment No. 39.48) and following sterile saline injection (experiment No. 39.2) are compared.

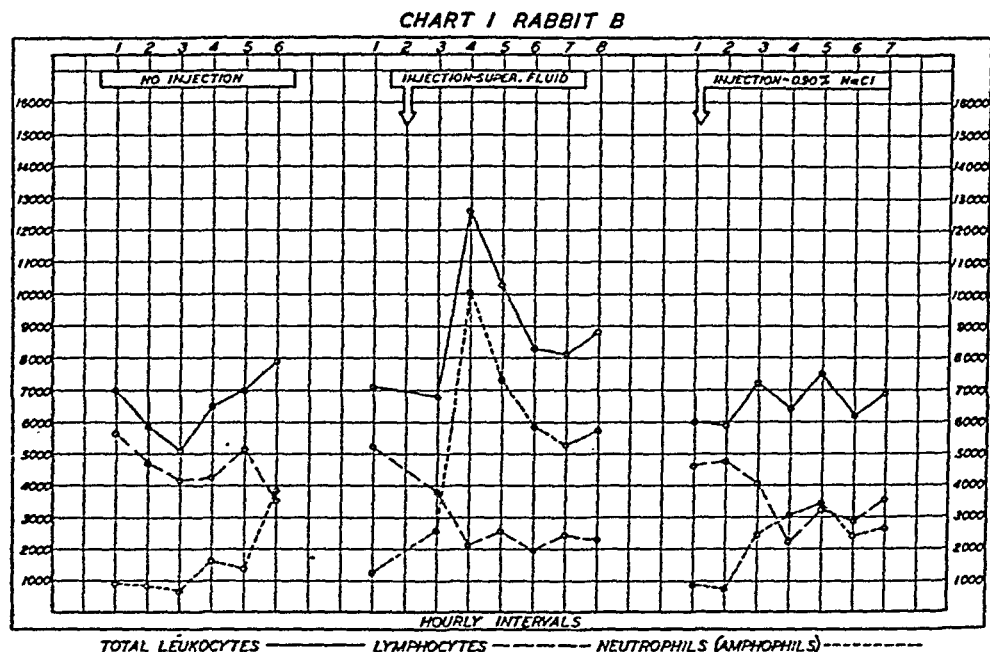


Chart 1 clearly indicates the sharply peaked neutrophilic leukocytosis which occurred 2 hours following the injection of supernatant fluid. The rise in total leukocyte count was apparently due entirely to the rapid increase in neutrophils, and the subsequent variations in the total leukocyte count reflected similar neutrophil variations.

Table I shows similar neutrophilic leukocytoses following intravenous injections of supernatant fluid into rabbit F. With this animal the neutrophil peak occurred $2\frac{1}{2}$ hours after injection in experiment No. 39.47 and 2 hours after injection in experiment No. 40.26. The increases in neutrophil percentages over respective periods of 3 and 4 hours were from 20 to 63 and from 17 to 54. The increase in absolute number of neutrophils was from 1,500 to 7,371 in experiment No. 39.47 and from 1,139 to 5,670 in experiment No. 40.26. The normal absolute neutrophil numbers, at times corresponding to the times of each neutrophil peak, were 1,513 and 2,037 respectively. While the neutrophilic leuko-

of the results. Hourly leukocyte variations of each animal were determined previous to any experimental procedures involving it and, as far as possible, the same times of counting were maintained when the leukocytes were studied after the animal had been injected. In all experiments, leukocyte counts were made at approximately hourly intervals through the sixth hour following injection. In 11 of the 13 experiments, determinations were made also 24 to 31 hours after injection.

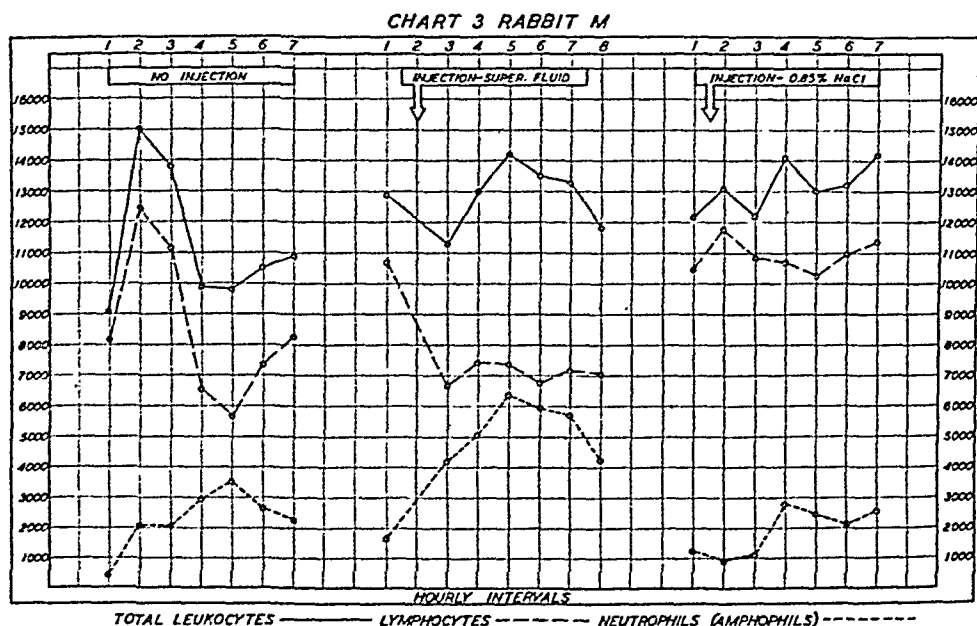
RESULTS

All results for each of the 9 rabbits are presented in Table I. Hourly total leukocyte counts and neutrophil (amphophil) and lymphocyte percentages are shown for each experimental procedure performed on each rabbit. Thus, the leukocyte picture of rabbit B was studied twice without injection (experiments Nos. 39.10 and 39.39), twice following an intravenous injection of 5 cc. of supernatant fluid from a sterile exudate (experiments Nos. 39.48 and 40.22), and once following an intravenous injection of 5 cc. of 0.9 per cent sterile sodium chloride solution (experiment No. 39.2).

With rabbit B, both intravenous injections of supernatant fluid were followed in 2 hours by absolute neutrophilic leukocytoses which were maintained for at least 6 hours after injection. The percentage of neutrophils increased in 3 hours from 18 to 80 in experiment No. 39.48, and from 8 to 78 in experiment No. 40.22. During these 3 hours the absolute number of neutrophils increased from 1,278 to 10,080 in the former experiment, and from 504 to 7,254 in the latter. The absolute number of neutrophils found in the circulating blood of this animal without injection, at the time of day corresponding to the time of the neutrophil peaks, was 663 in one experiment and 1,334 in the other. The increase in the total leukocyte count, although not marked in either instance, appeared to be definitely greater than the normal hourly total leukocyte fluctuations of this animal.

Although it may seem that the lymphocytes of rabbit B decreased almost as much as the neutrophils increased following injection in experiment No. 39.48, this lymphocytic decrease is no greater than the lymphocytic decrease which normally occurred in this animal during the same period of the day without injection.

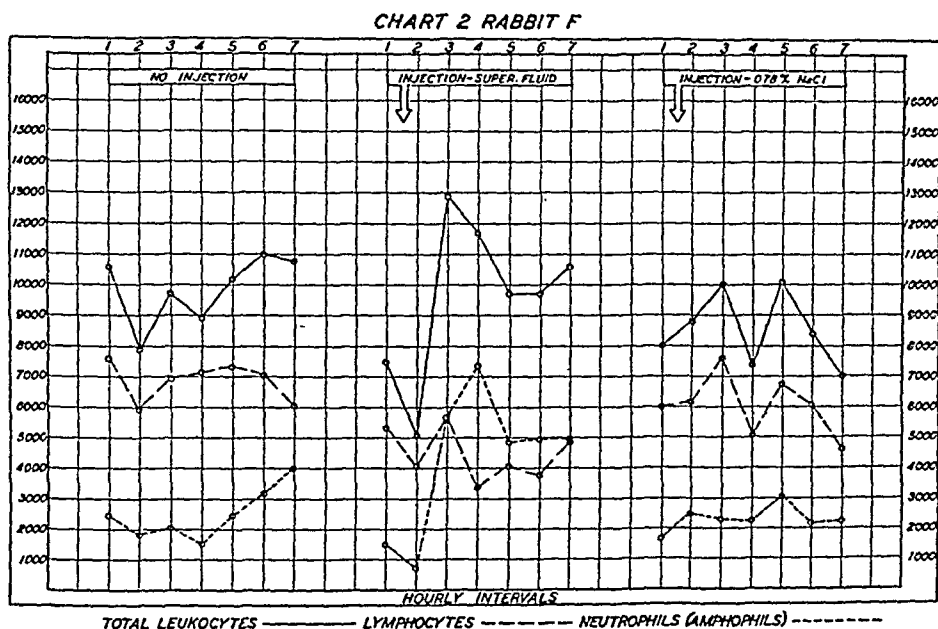
lowing the intravenous injection of supernatant fluid into rabbit M (experiment No. 40.10), a peaked neutrophilic leukocytosis occurred. During a 4 hour period the percentage of neutrophils increased from 13 to 45 and the absolute number of neutrophils rose from 1,677 to 6,390. This neutrophil peak was not as sharp as those of rabbits B and F in Charts 1 and 2 respectively, but it



occurred at approximately the same time after injection and was of sufficient magnitude to exceed considerably the normal absolute number of neutrophils at a corresponding hour of the day (2,970). No absolute neutrophilic leukocytosis occurred in this animal after the injection of supernatant fluid.

The results obtained with the remaining rabbits are tabulated in Table I. An absolute neutrophilic leukocytosis occurred in 5 of the 13 experiments (Nos. 39.47, 39.48, 39.49, 40.11 and 40.22), and was suggestive in another experiment (No. 40.26). However, the absolute numbers of neutrophils increased in all experiments following the injection of supernatant fluid. The average increase in absolute numbers of neutrophils from before supernatant fluid injection to the time of each neutrophil peak was 6,086; the corresponding average increase in neutrophil percentage was 42. The average increase in absolute numbers of neutrophils in the same animals without injection, during this same period of the day, was 1,389 and the corresponding average increase in neutrophil

cytosis was sharply peaked in both these experiments, an absolute neutrophilic leukocytosis occurred only in experiment No. 39.47. Chart 2 compares the leukocyte curves of rabbit F without injection (experiment No. 39.33), following supernatant fluid injection (experiment No. 39.47) and following sterile saline in-



jection (experiment No. 40.30). It is evident that the hourly absolute number of lymphocytes tended to remain fairly constant in all experiments performed on this animal.

After comparison of the leukocyte curves for the three experiments performed on rabbit M (Table I and Chart 3), it is evident that this animal always had a total leukocyte level of 10,000 to 15,000 per cu. mm. through the periods of counting. This has been confirmed by repeated leukocyte examinations of this animal without injection at approximately the same time of day on different days. Chart 3 shows that the sudden variations in the total leukocyte count, which occurred when the animal was examined hourly without injection (experiment No. 40.6), apparently were due to similar variations in the absolute number of lymphocytes. The absolute number of neutrophils increased slightly during the period of counting without injection, consistent with the hourly neutrophil variations of normal rabbits. Three hours fol-

Rabbits K, M and N showed the greatest fluctuation in absolute numbers of neutrophils of any of the 9 rabbits studied without injection. However, the neutrophil increases of rabbits K, M and N following supernatant fluid injection reached levels comparable to the neutrophil increases following such injection in the other 6 animals. Furthermore, Chart 3 shows that a neutrophil peak occurred 3 hours after rabbit M had received supernatant fluid. A similar neutrophil peak occurred 2 hours after supernatant fluid injection in rabbit N, and 3 hours after injection in rabbit K. At the times of these peaks the absolute numbers of neutrophils present in the circulating blood of these 3 animals were considerably in excess of the absolute number of neutrophils present in the blood of these animals without injection at times comparable to those of the neutrophil peaks.

Table II shows for all experiments the absolute numbers and percentages of neutrophils before injection, at the neutrophil peak, and without injection at corresponding times. The number of hours after injection that each neutrophil peak occurred is also indicated. It is evident that the neutrophil peak occurred most frequently (in 12 of the 13 experiments) at the second or third hour after injection. A secondary neutrophil peak occurred in 2 experiments (Nos. 40.9 and 40.24).

The duration of the exudate from which each supernatant fluid fraction was obtained is also indicated in Table II. In this series of 13 experiments, no differences were noted between the leukocyte counts of the 2 experiments with supernatant fractions of exudates of $5\frac{3}{4}$ hours' duration and the leukocyte counts of the 11 experiments with supernatant fractions of exudates of from $9\frac{1}{2}$ to 12 hours' duration.

In 4 of the 13 experiments, normal total and absolute leukocyte levels were reached 6 hours after injection. In the remaining 7 experiments in which leukocyte examinations were made more than 6 hours after injection, normal total and absolute leukocyte counts were present 24 to 31 hours after injection.

Five of the 9 rabbits (B, F, I, M and N) received an intravenous injection of 5 cc. of sterile sodium chloride solutions having chloride concentrations the same as those of the supernatant fluid fractions of the centrifugalized exudates. Charts 1, 2 and 3 indicate that the leukocyte pictures following sterile saline injec-

percentage was 10. In every experiment, the absolute numbers of lymphocytes, large mononuclear cells, eosinophils and basophils showed insignificant variations which were no greater than the hourly leukocyte variations of these animals, examined without injection.

VARIATIONS IN ABSOLUTE NUMBERS OF NEUTROPHILS (AMPHOPHILS)

□ SUPERNATANT FLUID INJECTION ▨ WITHOUT INJECTION

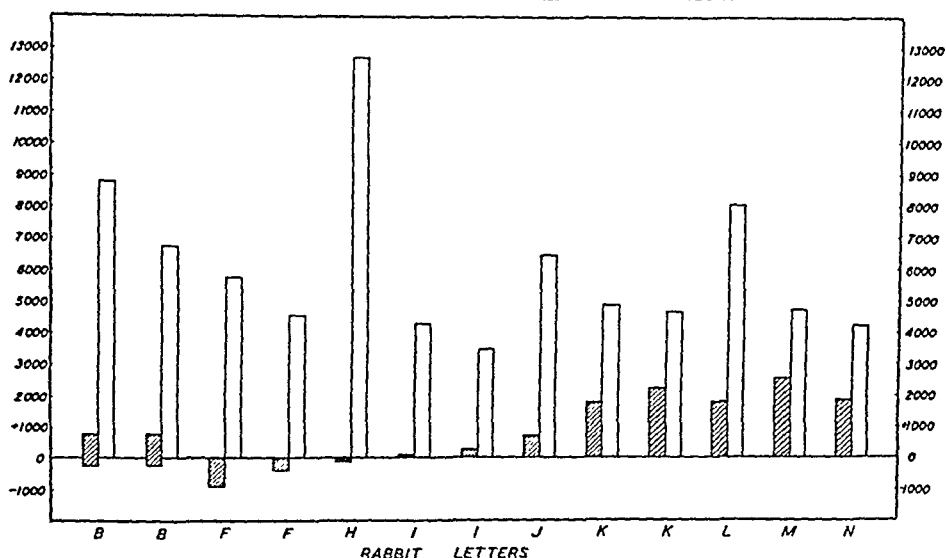


CHART 4. Variations in absolute numbers of neutrophils (amphophils) following supernatant fluid injection and without injection in the same animals. The blank column labelled "supernatant fluid injection" indicates the increase in absolute numbers of neutrophils (amphophils) which occurred from the time previous to the injection of supernatant fluid to the time of the neutrophil peak after the injection. The diagonally-crossed column labelled "without injection" indicates the increase or decrease in the absolute numbers of neutrophils (amphophils) which occurred in the same animals during a corresponding period of the day.

Chart 4 compares the variations in absolute numbers of neutrophils, from a time previous to injection to the time of the neutrophil peak following supernatant fluid injection, of each of the 9 rabbits, with the neutrophil variations of the same animals without injection during a corresponding period of the day. Except for 3 animals (K, M and N), the neutrophil increases following the injection of supernatant fluid considerably exceeded the normal neutrophil fluctuations during corresponding periods of the day in each case. In 3 animals (B, F and H), the absolute numbers of neutrophils decreased without injection during a period of the day comparable to the interval from before injection to the neutrophil peak.

tions of rabbits B, F and M respectively showed variations consistent with the fluctuations present in each of these animals without injection. Similar leukocyte fluctuations followed the sterile saline injection of 2 additional animals. In each of these 5 rabbits, the leukocyte variations following a control injection of sterile sodium chloride solution were no greater than the variations which occurred in the same animals studied without injection during a corresponding period of the day.

It is evident from Table II and Chart 4 that the results with rabbit H differed somewhat from those of the other 8 animals. In the first place, this animal, when examined without injection (experiment No. 39.40), had a relatively high percentage and absolute number of neutrophils. Following the intravenous injection of supernatant fluid from a sterile exudate (experiment No. 39.49), a peaked absolute neutrophilic leukocytosis occurred, the height of the neutrophilic increase being reached 3 hours after the injection. Leukocyte examinations made 4, 5, 6, 12, 31, 53, 96, 120 and 145 hours after the injection showed that normal leukocyte levels had not been reached at any time. Repeated examinations of this rabbit failed to reveal any abnormalities. The rectal temperature was consistently normal. The animal was killed 145½ hours after the supernatant fluid injection. An immediate autopsy showed purulent right otitis media. We feel that this infection was a possible cause of the atypical leukocyte counts obtained in this experiment.

DISCUSSION

Various agents are known to produce polymorphonuclear neutrophilic leukocytosis. Among these may be mentioned bacteria and their products,⁷ certain chemicals and drugs (such as histamine and epinephrine), nucleoproteins and some of their components, and various protein materials.⁸ The mechanisms of action of these various leukocytosis-producing substances is a matter of some controversy and will not be considered in this report.

A number of substances have been postulated to be responsible for the polymorphonuclear neutrophilic leukocytosis of acute inflammation. Acute inflammation may be accompanied by marked polymorphonuclear leukocytosis with or without bacterial infection being present. As bacterial products *per se* are known to

TABLE II
Characteristics of Neutrophilic Leukocytoses in 13 Experiments

Rabbit letter	Experiment number	Duration of exudate	Absolute numbers of neutrophils (amphophils)			Neutrophil (amphophil) percentages					Absolute neutrophilic leukocytosis	Return to normal level (hours after injection)
			Before injection	Corresponding hour without injection	At neutrophil peak	Corresponding hour without injection	Before injection	Corresponding hour without injection	At neutrophil peak	Corresponding hour without injection		
B	39.48	10 1/4	1278	910	10080	663	18	13	80	13	+	31
B	40.22	5 3/4	504	910	7254	663	8	13	78	13	+	Undetermined
F	39.47	9 1/2	1500	2438	7371	1513	20	23	63	17	+	26
F	40.26	12	1139	2438	5070	2037	17	23	54	21	?	6
H	39.49	10 1/4	738	3362	13440	3237	9	41	42	39	+	†
I	40.1	10 1/2	1008	1342	5264	1414	7	11	47	14	+	6
I	40.24	12	1176	1342	4620	1620	8	11	30	18	-	6
J	40.2	10 1/2	1270	1953*	7700	2646	10	21	55	27	-	25
K	40.8	10 1/4	1456	2625	6292	4386	13	21	52	43	-	30
K	40.23	5 3/4	2300	2625	6936	4828	20	21	68	34	-	Undetermined
L	40.9	10 1/4	3224	6004	11256	7776	26	38	67	48	-	30
M	40.10	10 1/4	1677	455	6390	2970	13	14	45	30	-	24
N	40.11	10 1/4	1562	676	5777	2508	22	13	53	38	+	6

* Approximate.

† Did not approach normal level 145 hours after injection; autopsy revealed otitis media.

tative analysis of these substances is one obstacle to be overcome in such a study of nuclear material. According to Sabin,¹⁶ nuclear material derived from constant breakdown of tissue may regulate the normal number of polymorphonuclear leukocytes in the circulating blood.

The suggestion has been made that histamine or histamine-like substances, such as the hypothetical H-substance of Lewis,¹⁷ may produce the polymorphonuclear neutrophilic leukocytosis accompanying acute inflammation. Moon, Lieber and Kennedy¹⁸ reviewed earlier studies of the effects of histamine on leukocyte counts and found "inconclusive evidence" that a relationship existed between histamine and leukocytosis either in animals or in man. Following the intravenous injection of cats with histamine phosphate in doses of 1 to 2 mg., they regularly observed absolute polymorphonuclear neutrophilic leukocytoses within several hours after injection, with peaks 2 to 4 hours after injection and a return to normal leukocyte levels within 24 hours. However, Menkin,¹⁴ who extracted histamine from exudates of dogs by the method of Barsoum and Gaddum,¹⁹ reported that the intravenous injection of histamine in amounts equivalent to the amounts recovered from such exudates failed to induce leukocytosis in dogs. In another report, Moon²⁰ stated that sufficiently large amounts of histamine might be released from extensive areas of injured tissue to be a possible factor in the production of leukocytosis. However, he also considered that some substance, apparently not histamine, was released from injured cells and attracted leukocytes to an area of injury. Moon believed that this substance was perhaps also effective in the production of systemic leukocytosis.

Danzer²¹ prepared isotonic saline extracts of various normal rabbit organs and tissues, including muscle, liver, testicle, brain and hemolyzed erythrocytes. He reported that the subcutaneous or intravenous injection into rabbits of 3 to 5 cc. of such extracts was followed in 5 hours by a marked polymorphonuclear (amphophil) leukocytosis, lasting several days to a week. Danzer considered that this leukocytosis was caused by proteins contained in the extracts.

Following the intracutaneous injection of hemolytic streptococci into rabbits, Nettleship²² observed that absolute polymor-

cause leukocytosis, it is possible that the leukocytosis of acute inflammation often may be the result of action of more than one substance.

Many observations have shown that polymorphonuclear neutrophilic leukocytosis may occur after the injection of nucleic acid or some of its derivatives.⁹⁻¹² Intravenous injections into rabbits of approximately 1 gm. of nucleic acid or its salt, sodium nucleinate, frequently are followed by a preliminary period of leukopenia lasting approximately 5 or 6 to 24 hours. This leukopenic phase may be followed by an absolute polymorphonuclear (amphophil) leukocytosis which reaches its peak 7 or 8 to 24 hours after injection, with a return to normal leukocyte levels 3 or 4 days following the injection. A more rapidly developing absolute polymorphonuclear (amphophil) leukocytosis, with a peak several hours after injection and a return to normal leukocyte levels 8 to 48 or more hours after injection, frequently follows the intravenous injection into rabbits of approximately 1 gm. of nucleic acid derivatives, such as adenine or guanine nucleotides.^{12,13} Doan, Zervas, Warren and Ames¹² reported a similar prompt absolute polymorphonuclear (amphophil) leukocytosis following intravenous injection of approximately 1 gm. of sodium nucleinate into splenectomized rabbits. The initial leukopenic phase was absent in these accelerated leukocytoses.

The relatively prompt leukocytic response which follows the intravenous injection of fractions of exudates is unlike the more slowly developing leukocytosis which follows intravenous injection of nucleic acid or sodium nucleinate, but does resemble the more rapidly developing leukocytosis which occurs after intravenous injection of nucleotides. Menkin¹⁴ has reported similar observations in connection with studies of exudates in dogs and stated that the possibility is not precluded that inflammatory exudates may contain considerable quantities of nucleotides.

While a nucleotide has been isolated from human blood,¹⁵ there have been no reports of determinations of the amounts of nucleic acid or its derivatives in the circulating blood in the presence of acute inflammation. We would expect an increase above a normal range if these substances were present at an area of acute inflammation and were carried in the circulating blood to effect leukocytosis. The present complexity of methods for the quanti-

of 10 cc. of supernatant fluid from such an exudate was reported to have been followed in 4 hours by a relative polymorphonuclear (amphophil) leukocytosis in the injected animal.

Our results seem to indicate that some substance or substances present in the supernatant fluid fractions of centrifugalized rabbit exudates of 5¾ to 12 hours' duration produced the neutrophilic leukocytoses observed in our experiments. The regular occurrence of the peaks of the neutrophilic leukocytoses 2 to 3 hours following the intravenous injection of supernatant fluid suggests that the same substance or substances may have produced the leukocytosis in all experiments.

The leukocytosis-producing property of these supernatant fluid fractions apparently did not depend on their concentrations of sodium chloride. Intravenous injections of equivalent amounts of sterile sodium chloride solutions, of the same sodium chloride concentrations as the supernatant fluids, into the same experimental animals failed to show neutrophilic leukocytoses when leukocyte counts were made during corresponding periods of the day.

Apparently bacteria and their products also may be eliminated as possible causes for the neutrophilic leukocytoses observed in these experiments, as repeated bacteriological studies yielded negative results.

At the present time we cannot make any statements regarding the physical and chemical nature of the leukocytosis-producing material or materials with which we are dealing in these experiments. Protein material undoubtedly is present in these supernatant fluid fractions, because clotting has occurred in those which have been kept for several days to several months.

SUMMARY

Sterile peritoneal exudates were recovered 5¾ to 12 hours after the intraperitoneal injection into rabbits of sterile 0.9 per cent sodium chloride solution. These exudates were centrifugalized and their supernatant fluid fractions retained. Thirteen experiments were performed, in which 5 rabbits received 1 intravenous injection and 4 rabbits received 2 intravenous injections (at intervals of 58 to 106 days) of 5 cc. of sterile fresh supernatant fluid from these sterile peritoneal exudates.

phonuclear (amphophil) leukocytoses occurred within 1 hour and were maintained for 1 to 3 weeks. From correlation of the times of appearance of necrosis and leukocytosis, Nettleship postulated that substances released from the cytoplasm of necrosing polymorphonuclear leukocytes appeared to diffuse into the blood stream and cause the leukocytosis. He then prepared sterile water extracts of polymorphonuclear leukocytes of rabbits (amphophils) from 48-hour sterile aleuronat pleural exudates and from centrifugalized blood, and sterile water extracts of rabbit bone marrow. Nettleship²³ reported that the intravenous injection into rabbits of 4 to 10 cc. of such extracts was followed in 2 hours by marked polymorphonuclear (amphophil) leukocytoses which lasted several days. Nettleship considered that the leukocytosis-producing principle present in these extracts of hematopoietic tissue was probably protein in nature, and termed this substance "leucogen." He reported that the intravenous injection of sterile water extracts of nonhematopoietic rabbit tissues was not followed by such polymorphonuclear (amphophil) leukocytosis.

Menkin²⁴⁻²⁶ produced pleural exudates of from 1 to several days' duration in dogs by the intrapleural injection of turpentine. He described the isolation from such exudates of two nitrogenous substances, which he termed "leukotaxine" and a "leukocytosis-promoting factor," both presumably liberated in areas of acute inflammation. Menkin considered "leukotaxine" to be a polypeptide which was capable of increasing capillary permeability and of inducing prompt polymorphonuclear leukocytic migration at the site of an injury. The "leukocytosis-promoting factor" has been described^{14,26} as being associated with the globulins and capable of inducing absolute polymorphonuclear neutrophilic leukocytoses in dogs when injected intravascularly. Menkin stated that "it is highly improbable that the effect of exudates on the leukocyte level might in any way be referable to a direct action of turpentine *per se* contained in exudates." Exudative material from a burned area of skin on the leg of a dog was also reported to have been followed by leukocytosis when injected into the blood stream.²⁷

Ponder and Macleod,³ using intraperitoneal saline infusions, produced peritoneal exudates of approximately 18 hours' duration in rabbits. The intraperitoneal injection into another rabbit

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Hourly leukocyte examinations in all 13 experiments showed that in each case a neutrophilic leukocytosis occurred a few hours after injection. In 5 of the 13 experiments there was an absolute neutrophilic leukocytosis. The peak of the neutrophilic leukocytosis occurred 2 or 3 hours after injection in 12 experiments and 4 hours after injection in the remaining experiment. There was a return to normal total and differential leukocyte counts 6 to 31 hours after the supernatant fluid injections.

Leukocyte examinations, made at hourly intervals during the same period of the day, of the same 9 rabbits without injection and of 5 of these 9 rabbits following intravenous injections of 5 cc. of sterile sodium chloride solutions, showed no neutrophilic leukocytosis in any experiment. The chloride concentrations of these sterile saline solutions were equivalent to the chloride concentrations of the supernatant fluids which were injected.

CONCLUSION

These results seem to indicate that some substance or substances present in the supernatant fluid fractions of centrifugalized saline-induced rabbit peritoneal exudates of 5¾ to 12 hours' duration can produce neutrophilic leukocytoses when injected intravenously into other rabbits.

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It seemed of considerable interest to test whether these tumor-growth-inhibiting properties of yeast could be made available in extract form and used successfully in spontaneous malignant tumors.

CLASSIFICATION OF EXTRACTS

For the sake of brevity we shall refer to the different extracts which were used in the treatment of spontaneous tumors in the following manner:

- S₁: Healed splenic extract prepared from spleens of mice previously healed of Sarcoma 180 by a concentrated splenic extract.
- S₂: Splenic extract purified by dialysis and fractionation from concentrated splenic extract.
- S₃: Splenic extract prepared by immediate cold extraction.
- Y: Yeast extract.

DESCRIPTION OF EXTRACTS

Extract S₁. Healed splenic extract was prepared in the following manner: The mice were killed with ether and their spleens were immediately removed, weighed, frozen on blocks of dry ice and ground in a mortar with sand. Distilled water and chloroform were added in the proportion of 50 cc. of water and 5 cc. of chloroform to 5 gm. of spleen. The mixture was shaken for about 8 hours in a shaking machine and kept in an icebox for at least 48 hours. The proteins were precipitated with 10 per cent trichloroacetic acid. The mixture was centrifugalized for about 15 minutes at the rate of 3000 r. p. m. The supernatant, light yellowish fluid was passed through a filter paper and concentrated at 60° C. *in vacuo*, until 1 cc. of the extract represented 1 gm. of fresh spleen. The acid extract was neutralized with sodium hydroxide using litmus paper as an indicator, and filtered through a Seitz filter No. 3. Merthiolate (1:200,000) was added and the extract was collected in sterile ampules.

Extract S₂. The concentrated spleen extract was treated by continuous dialysis *in vacuo* at reduced temperature (40° to 50° C.) in the apparatus of Hanke and Koessler⁴ for a period of

THE TREATMENT OF SPONTANEOUS BREAST ADENOCARCINOMAS IN MICE WITH EXTRACTS OF SPLEEN OR YEAST *

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INTRODUCTION

It is not possible, in a brief presentation of this subject, to review previous work done by others who have attempted to heal spontaneous malignant tumors in mice or other animals. Furthermore, such a review would present an almost uninterrupted series of failures extending over about 30 years unless radiotherapy was employed. Occasionally, complete regressions have been reported in a small group of animals. However, these scattered reports either dealt with a very small number of animals or lacked confirmation when the experiments were repeated.

Experiments with any new form of cancer treatment should be started on transplanted tumors, as large numbers of animals with tumors of this type are easily obtainable. After its therapeutic efficacy has been proven on transplanted tumors, the new procedure should be tested on spontaneous tumors, for if complete regressions are obtained in transplanted tumors the same therapy *may* show results in spontaneous tumors. However, if a therapeutic attempt fails in transplanted tumors, spontaneous tumors will not respond to that treatment.

Our work has followed this sequence. After Lewisohn¹ in 1938 had reported 60 per cent cures among 281 mice with sarcoma 180 with a concentrated splenic extract, extracts of spleen in a variety of preparations were tested in spontaneous breast adenocarcinomas in mice (Lewisohn, Leuchtenberger and Laszlo²). Maisin³ reported that by adding baker's yeast to the food of his animals he was able to reduce the incidence of malignant tumors in mice which had been treated with benzpyrene.

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CHEMICAL AND PHARMACOLOGICAL DATA

Experiments, carried out in this laboratory during the past 1½ years, have dealt with the chemical and pharmacological aspects of the following problems:

1. *Characterization of Concentrated Spleen Extract.* The concentrated spleen extract contained 14 per cent total solids. The dry residue contained 16.1 per cent nitrogen and 9.3 per cent ash. The extract had a P_H of 6.2. The minimum lethal dose upon subcutaneous administration was 0.5 cc., corresponding to 70 mg. dry substance, for a 20 gm. mouse; and 0.35 cc., corresponding to 49 mg. dry substance, upon intravenous injection. This toxicity was at least partly caused by concomitant vaso-active substances, the presence of which is irrelevant for the tumor-inhibitory effect.

2. *Fractionation of the Concentrated Spleen Extracts.* Upon dialysis, the tumor-inhibitory activity was found in the dialysate. This was concentrated to about one half its volume *in vacuo*, then precipitated with twenty times its volume of absolute alcohol. The precipitate was washed repeatedly with 95 per cent alcohol and with absolute alcohol, and upon re-precipitation the activity remained in the precipitate. This precipitate corresponded to 15 to 20 per cent of the original solids. Its toxicity was reduced one third to one fourth as compared with the toxicity of the original extract. The vaso-active factors were largely eliminated and the tumor regression, caused by these purified extracts, ran its course without hemorrhagic phenomena.

3. *Extract from 'Fresh Spleen in the Cold.* Fresh calves' spleens, obtained in ice from the slaughter-house, were minced under ice-cold trichloroacetic acid. The filtrate, 1 cc. of which corresponded to 1 gm. of spleen, contained the tumor-inhibitory principle. Therefore, under these precautions, the yield of the principle may be improved so as to make a subsequent concentration of the extract unnecessary.

4. *Yeast Extracts.* Aqueous yeast extracts proved tumor-inhibitory. The extracts were dialysed, precipitated with absolute alcohol, as in the case of the spleen extracts, and the precipitate purified, washed and re-precipitated as before. Activity was again found in the final alcohol precipitate. The results of chemical analysis and of toxicity tests are given in Table I.

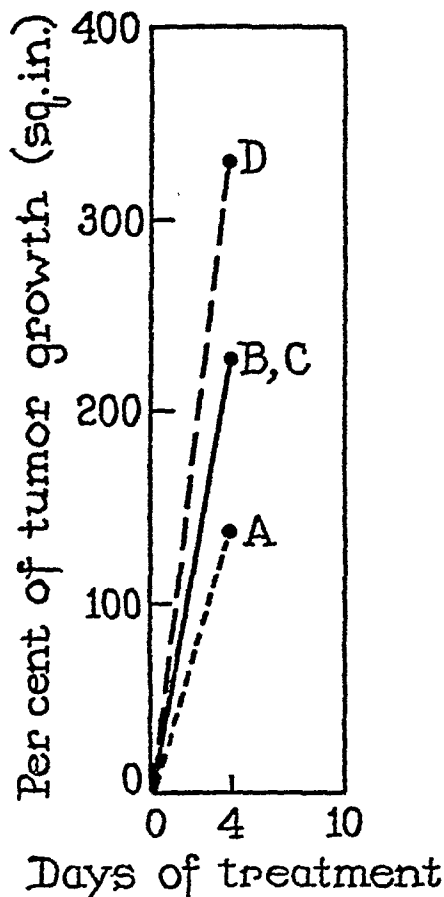
48 hours. One hundred cc. of the extract was dialysed under a layer of toluene against 600 cc. of water which was changed three times. In pilot experiments it was found that the dialysate, after this treatment, was free of sodium chloride and failed to give a biuret reaction. Both the dialysate and the nondialysable residue were concentrated to the original volume *in vacuo* at temperatures below 60° C.

The dialysate was precipitated with absolute alcohol to give a final concentration of 95 per cent. Excessive concentration of the dialysate before alcohol precipitation should be avoided and we confined ourselves to a concentration of 40 per cent of the original volume. The precipitate was kept for 24 to 48 hours in an icebox, after which the precipitate was separated by decantation from the strongly yellow, alcoholic, supernatant fluid. The precipitate was washed several times with 95 per cent alcohol and centrifugalized. Finally it was dissolved in the smallest possible amount of water and again treated with alcohol as before. The combined precipitates were dissolved as completely as possible in water, the solution freed of alcohol *in vacuo* and the volume reduced to the volume of the original extract. The alcoholic supernatant fluids were collected, the alcohol removed *in vacuo*, and the residue brought again to the starting point.

Extract S₃. Fresh, refrigerated spleens obtained from the slaughter-house were minced in trichloroacetic acid and the suspension kept for 4 hours at temperatures between 0° and 4° C. with frequent agitation. The filtrate obtained was brought *in vacuo* below 60° C. to the desired concentration of 1 cc. per 1 gm. of fresh weight. The extract was neutralized before injection and in some instances the trichloroacetic acid was extracted with ether before use.

Extract Y. Of fresh brewer's yeast, 2500 gm., corresponding to 500 gm. of dry substance, was washed several times with distilled water. The yeast was boiled for 7 minutes with 9000 cc. of distilled water to which 0.9 cc. of glacial acetic acid had been added. The extract was filtered through paper pulp and concentrated *in vacuo* at 70° C. to 1500 cc. It was precipitated with 1500 cc. of absolute alcohol. The filtrate was concentrated *in vacuo* at 70° C. to 900 cc.

animals (Text-Figure 1). We are now engaged in the statistical treatment of this aspect of the problem.



TEXT-FIGURE 1. Effect of different extracts on tumor growth after 4 days of treatment. C=control. A=1.5 per cent solution of the alcohol insoluble fraction. B=1.5 per cent solution of the alcohol insoluble fraction boiled 3 minutes with alkali. D=1.5 per cent solution of the alcohol insoluble fraction boiled 60 minutes with alkali.

TECHNIC

The technic (route, dose, frequency) of the injections has been discussed in detail in previous papers.^{1,2} The spleen extracts were given intravenously or intraperitoneally. The yeast extract was given intravenously, usually combined with a subcutaneous injection.⁵ The average dose for intravenous or intraperitoneal injection was 0.1 to 0.15 cc., whereas 0.5 cc. was given subcutaneously. Up to forty-three intravenous injections have been given to the same animal. Injections were given daily (in the majority of cases) or on alternate days.

BIOLOGICAL OBSERVATIONS

The material under consideration consisted of 363 mice with spontaneous breast tumors, in all of whom a biopsy was performed to establish the diagnosis. Whenever the slightest doubt existed as to the definitely malignant character of the tumor, the animal was excluded. Forty-one (11 per cent) proved to be nonmalignant. Additional proof that the diagnosis of malignancy was justified in the remaining 322 mice can be found in the fact that lung metastases were present in 30 per cent of those which came to autopsy. The great importance of a biopsy of spontaneous tumors is evident because it is impossible to establish the malignant or nonmalignant character of these tumors by palpation.

TABLE I
Fractionation of Yeast Extract

Extract	Dry substance, per cent	N ₂ in per cent of dry substance	P in per cent of dry substance		Reducing power of the extracts calculated as glucose (Hagedorn-Jensen) in per cent of the total solids			Growth-factor in mg.*	P _H	Toxicity (lethal dose) in cc. and mg. by intravenous administration
			Inorganic	Organic	Direct	After 0.04 normal NaOH	Difference			
Original crude extract	6.82	8.51	—	—	6.82	6.12	0.70	—	5.42	0.15 cc. = 10.20 mg.
Dialysate	5.15	8.16	—	—	7.12	6.16	0.94	—	5.90	0.20 cc. = 10.30 mg.
Alcohol soluble fraction	1.95	12.72	0.10	1.50	13.66	13.66	0	0.122	5.02	0.45 cc. = 8.80 mg.
Precipitate	3.20	6.72	3.40	2.44	17.67†	13.47	4.20	0.023	6.60	1.10 cc. = 16.50 mg.

* Growth-factor = The smallest amount of extract in mg. of solids which supports the growth of *Bacillus influenzae* in autoclaved Difco medium.
 † Reducing power after acid hydrolysis, 25.07.

The digestion of the precipitate with weak alkali on the boiling water bath destroyed part or all of the tumor activity, depending on the time of boiling and the concentration of alkali, and may even convert this activity into a tumor-growth-promoting factor.* The inhibitory activity disappeared, according to our experiments, in parallel with the disappearance of the ability of the same extracts to support the growth of bacteria on autoclaved media. The alcohol precipitate reduced alkaline potassium ferricyanide solution. This reducing power was partly destroyed by boiling with alkali, but the loss of tumor-inhibitory activity took place while the reducing power was still intact.

5. *Tumor Growth Curves as Preliminary Tests.* In a large number of transplanted tumors we have planimetrically measured the growth during a given period, and have compared area increment of extract-treated tumors with untreated controls. When using potent extracts we have found, even within the first week, a diminution of growth as compared with the controls. The amount of inhibition of growth may be used as an indicator for the potency of the extracts. This measure parallels, in many instances, the survival rate of the tumor-transplanted

* In recent experiments we have studied the effect of heat at various P_H levels on the growth-inhibiting properties of yeast extracts.

plained by the fact that treatment was discontinued immediately after the tumor had disappeared. Since our procedure has been changed and treatment has been continued even after complete disappearance of the tumors, we have not observed recurrences. However, the final decision on this important point must await the natural death of the 38 healed animals which are living at present.

Among the 11 recurrent tumors, 10 occurred apparently in the original location, whereas 1 was observed in an entirely different part of the body. It is impossible to say whether these 10 new tumors occurred in exactly the same location or in an adjacent breast. The mammary glands in a mouse are in such close proximity that an exact evaluation of this point is practically impossible.

All of the recurrent tumors have been completely resistant to treatment. We have not been able to influence their growth, even when they were small. Thus, whereas large primary tumors responded to treatment, the recurrent tumors in the same animal (even when they were of small size) continued to grow in spite of frequent intravenous injections. Apparently the tumor cells have become resistant to the treatment.

It might appear from these statistics that the percentage of healed animals was only 20 per cent (38 among 189 animals). However, the actual figures do not give a correct picture. Working in a new field, we experimented with a large number of different extracts or their fractions. Some of these were complete failures. For instance, in a considerable number of mice, necrosis of the tail was noted after a few injections and further treatment had to be discontinued. Other fractions proved to be very toxic. Furthermore, a severe, intercurrent infection killed a considerable number of animals which were practically healed and whose tumors possibly would have disappeared completely.

When using effective and nontoxic extracts we maintained the percentage of total regressions at approximately 30 per cent.

In a previous paper² we mentioned that in addition to the healed animals with complete regressions, 30 per cent of the mice showed a reduction in the size of the tumors at the time of death. If we were to include these animals, we would arrive at a total figure of 60 per cent in which the tumor growth was influenced favorably. However, we prefer not to include this latter group.

It is generally accepted that spontaneous tumors whose malignant nature is proven by biopsy do not disappear spontaneously. We felt that it was of great importance to establish whether biopsy alone might not influence the natural evolution of these tumors. No data on this question could be found in the literature. Sixty-eight tumor mice (60 from the Jackson Memorial Laboratory and 8 from the Rockland Farms) were used as controls, exposing them to biopsy only. Up to now we have not observed disappearance of the tumors in these controls, though some tumors showed temporary regression.

Twenty-eight animals were discarded for the following reasons: (1) death due to ether before biopsy was taken, (2) death during biopsy (in a number of instances pulmonary metastases were found at postmortem examination), (3) pregnancy, (4) cannibalism by other animals, (5) died without treatment or during the first 2 or 3 days.

Twenty-one animals are still under treatment. Sixteen other animals were treated with an immune spleen extract as described in a previous paper.² While 4 of these tumors disappeared temporarily, they all recurred. Therefore, these 16 cases are not considered in this report. Thus only 189 mice are the subjects of this presentation.

Among these 189 animals, 38 are healed at present. Of these, 27 were treated with various spleen extracts and 11 received yeast extract. There were 11 additional animals, treated with spleen extracts, in which a complete retrogression was first effected, but subsequently these animals showed recurrences (Table II). As stated previously,² we feel that these recurrences may be ex-

TABLE II
*Summary of Apparently Healed Animals, of Recurrences
and of Animals Remaining Healed*

Extract	Animals apparently healed	Animals with recurrences	Animals remaining healed
Splenic S ₁	22	7	15
" S ₂	7	1	6
" S ₃	8	3	5
" R ₁	1	0	1
	38	11 (29%)	27
Yeast	11	0*	11

* Up to March 1, 1940. Oldest healed animal: 72 days since complete macroscopic regression.

lines. Nicotinamide is not tumor-active. Possibly cozymase may prove to be a tumor-reducing substance.

SUMMARY

1. Thirty-eight spontaneous breast adenocarcinomas in mice, proven by biopsies, disappeared completely following treatment with spleen or yeast extracts.

2. The method of preparation of the different extracts is presented.

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DESCRIPTION OF PLATES

PLATE 48

FIG. 1. Mouse No. J.M. 404, healed by yeast extract. Lesion was proved to be carcinoma by microscopical examination. Treatment was started on December 23, 1939 and growth had disappeared by January 16, 1940. This animal received 12 intravenous and 10 subcutaneous injections of yeast extract.

FIG. 2. Mouse No. J.M. 402, healed by yeast extract. Neoplasm was proved to be carcinoma by microscopical examination. Treatment was started on December 23, 1939 and growth had disappeared by January 16, 1940. This animal received 11 intravenous and 10 subcutaneous injections of yeast extract.

We have, in a large number of cases, observed a temporary reduction in size during treatment with subsequent increase in growth. Thus we must not lose sight of the fact that these tumors might have started to grow again, if the animals had not succumbed. Furthermore, we have noted among the controls, marked transient reduction in the size of the tumors following simple biopsy. The only proper criterion is the complete and permanent disappearance of the tumor.

Of interest among the healed animals is the group of 5 cases (Table III) in which microscopic postmortem examination 149, 129, 93, 49 and 19 days after the mice were pronounced healed,

TABLE III
Number of Days Which Have Elapsed Since Tumors Were Healed
(Last Reading: March 1, 1940)

Animal No.	Extract	Days elapsed since complete regression of tumor	Animal No.	Extract	Days elapsed since complete regression of tumor
J.M. 210	S ₁	216	J.M. 362	Y	72
J.M. 199	S ₁	205	J.M. 350	Y	70
J.M. 228	S ₁	197	J.M. 310	Y	66
J.M. 207	S ₁	171	J.M. 307	S ₂	66
J.M. 153	S ₁	155 animal lost	J.M. 364	S ₁	65
J.M. 155	S ₂	149 no carcinoma*	J.M. 329	S ₂	55
J.M. 232	S ₃	133	J.M. 327	S ₂	51
J.M. 234	S ₃	133	J.M. 367	S ₁	49
J.M. 204	S ₃	132	J.M. 143	S ₁	49 no carcinoma*
J.M. 147	S ₁	129 no carcinoma*	J.M. 404	Y	44
J.M. 132	S ₁	120	J.M. 338	S ₃	41
J.M. 275	S ₁	108	J.M. 352	Y	40
J.M. 278	S ₃	108	R. 22	Y	39
J.M. 271	S ₁	108	R. 18	Y	38
J.M. 194	R ₁	104	R. 21	Y	35
J.M. 263	S ₁	102	J.M. 464	Y	26
J.M. 304	S ₂	102	J.M. 402	Y	23
J.M. 157	S ₂	93 no carcinoma*	J.M. 134	S ₁	19 no carcinoma*
J.M. 363	S ₁	81	R. 27	Y	12

Animals 134, 143, 147, 155, 157 died. Others are still living.

J.M.=Jackson Memorial Laboratory.

R.=Rockland Farms.

R₁=Concentrated spleen extract (100 gm. in 1 cc.) prepared in this laboratory and used in a few animals.

* As determined by postmortem microscopical examination.

failed to show a trace of cancer cells. They seem to offer additional proof that these cancers were healed by splenic extracts. The 11 animals healed with yeast extract (first treatment November 9, 1939) were all alive at the time of this report.

The fact that two different extracts (spleen and yeast) caused total regressions in these tumors, may help in the search for the active principle. We have started some investigations along these

PLATE 49

FIG. 3. Mouse No. R. 27, healed by yeast extract. Neoplasm was proved to be carcinoma by microscopical examination. Treatment was started February 5, 1940 and growth had disappeared by February 17, 1940. This animal received 8 intravenous and 7 subcutaneous injections of yeast extract.

FIG. 4. Control mouse No. R. 8, proved to have carcinoma by biopsy. Drawings to show progress of growth in this untreated animal between January 3, 1940 and February 23, 1940.

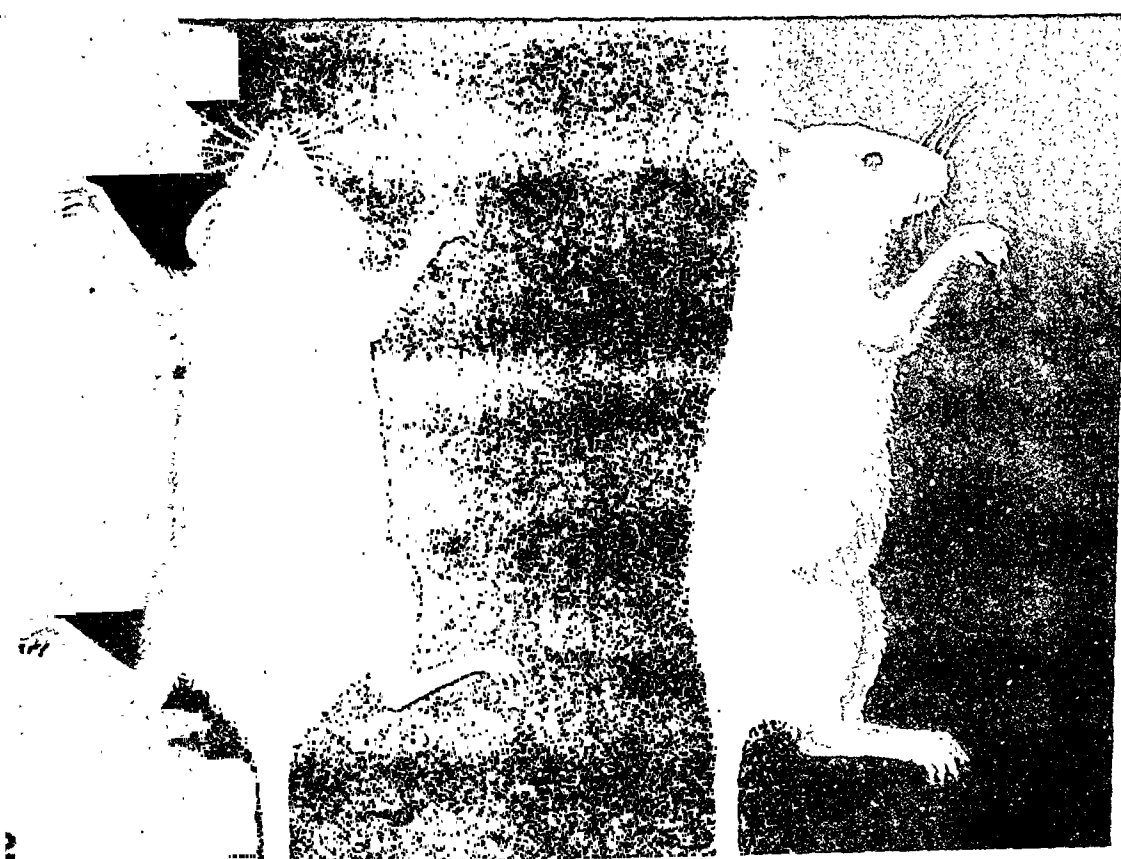
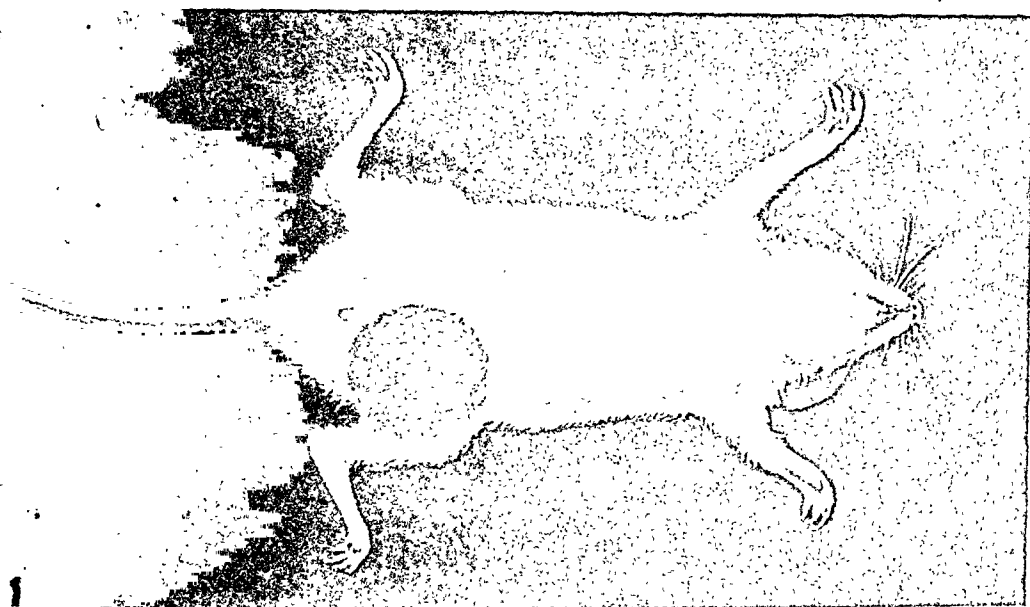
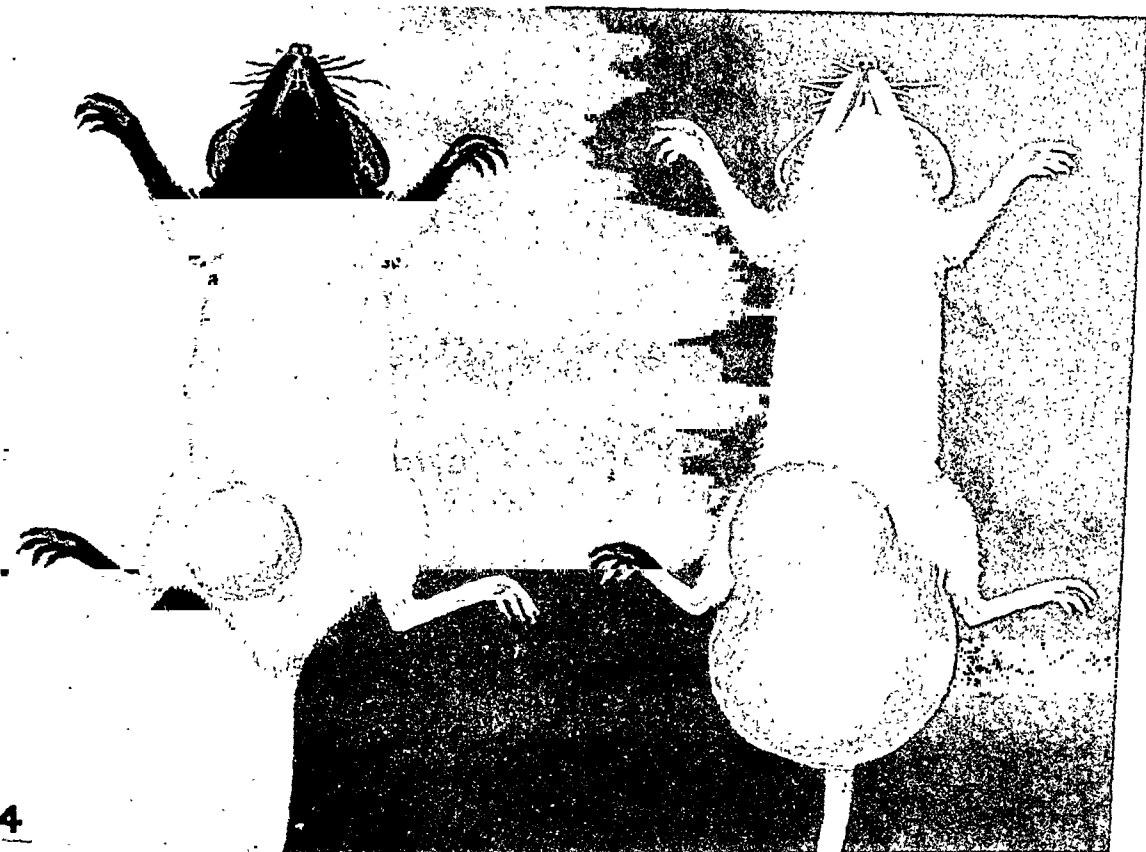
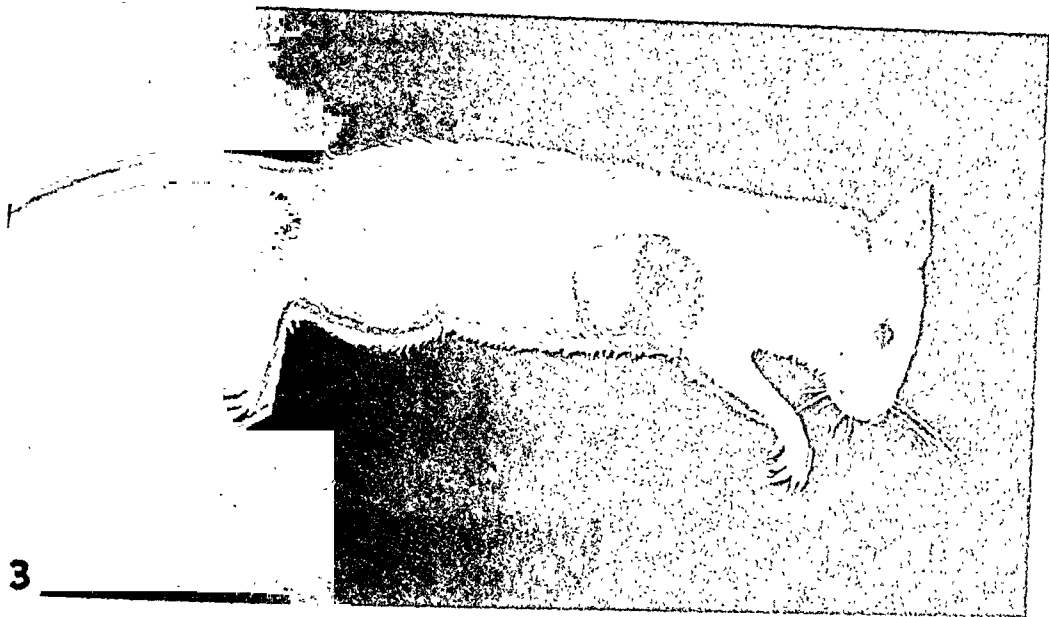
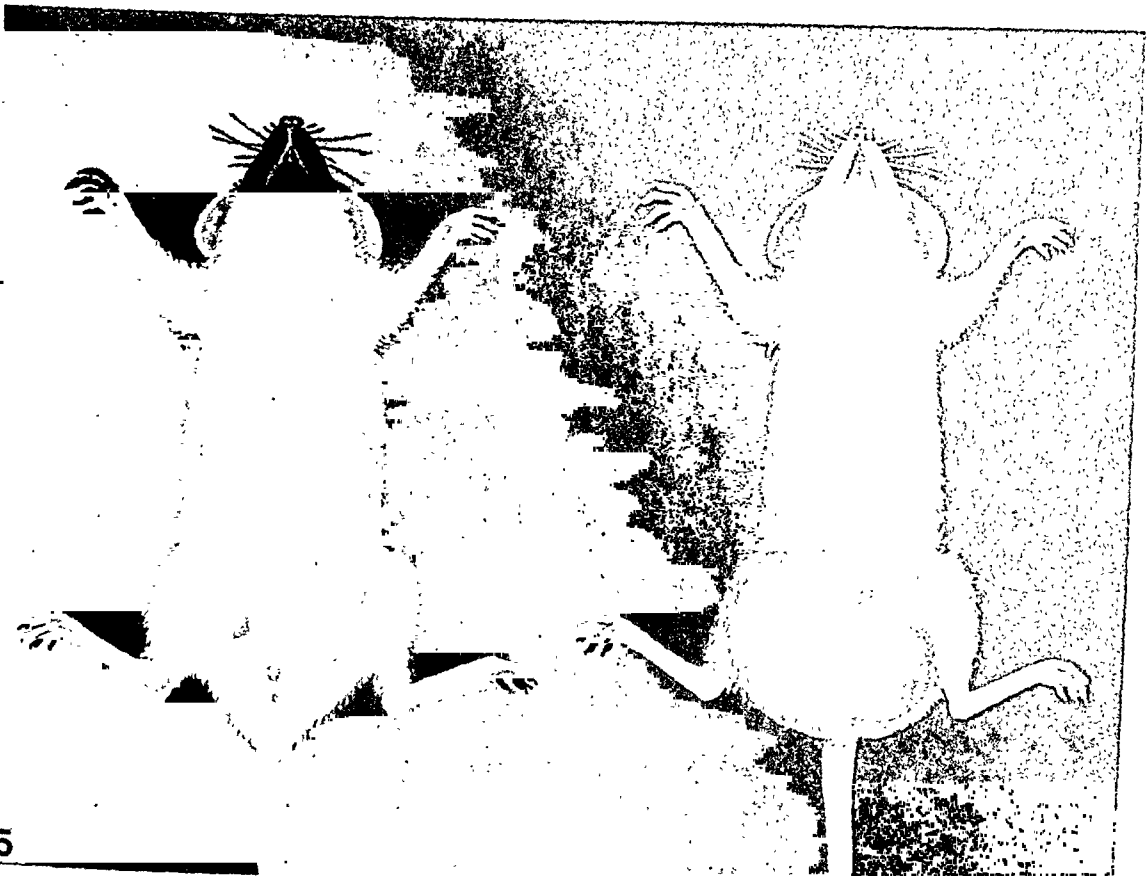


PLATE 50

FIG. 5. Control mouse No. R. 11, proved to have carcinoma by biopsy. Drawings to show progress of growth in this untreated animal between January 3, 1940 and February 23, 1940.





Lewisohn and Associates

Treatment of Adenocarcinomas in Mice

who seems to have been the first to postulate that the tumors began as organizing thrombi and underwent metaplasia into true neoplastic growths. Thorel quoted Czapek⁹ in support of the organizing-thrombus theory and concluded finally that, if the structures were true tumors, there were two possibilities for their explanation: (1) they arose from myxomatous metaplasia of a thrombus; and (2) they came from the myxomatous anlagen of the valves, rests of which either might persist in accordance with Ribbert's theory or revert in later life to an embryonic myxomatous state.

Brenner¹⁰ in 1907 collected 32 myxomas of the heart and added 1. Of these, 19 were in the left auricle, 10 on heart valves, 2 in the right ventricle and 1 beneath the epicardium at the apex. Of the 19 in the left auricle, 8 were on the septum, 6 above the mitral valve and 5 at various other points on the wall. Two of those which occurred on valves were on the mitral, 6 on the tricuspid and 2 on the pulmonic. Brenner believed the tumors to be true neoplasms, although he said they could not all be differentiated in the gross but had to be diagnosed microscopically; this could be done on their arrangement and cytology, and sometimes microchemically by the use of the mucicarmine reaction. Husten¹¹ in 1922 collected 71 polypoid tumors of the endocardium of the right heart and 17 of the left. He divided and classified them into organized thrombi, pseudotumors and true myxomas. He accepted only 9 from the right heart and 2 from the left as true myxomas. He cited six authors who had reported sarcomas but did not discuss them, since they were definitely malignant. Kirch¹² in 1927 added 12 myxomas, including 2 hemangiomyxomas, and concluded that the older views of Thorel and Czapek could no longer be held but that the growths were true neoplasms.

Other cases of polypoid tumors of the heart which we were able to review and which resembled ours in the gross included those of Binder,¹³ 1913 (sarcoma); Binder,¹⁴ 1927 (myxoma); Barnes and Yater,¹⁵ 1929; Jaleski,¹⁶ 1934; Fossel,¹⁷ 1936, 2 cases; and Müller,¹⁸ 1932, who reported 3 myxomas and 1 sarcoma.

Diebold¹ in 1930 was the first to separate the sarcomas of the heart from the myxomas. He collected 45 cases and prepared a table giving the authors, sites of the primaries and metastases, microscopic diagnoses and clinical notes. Of 34 cases upon which

PRIMARY FIBROMYXOSARCOMAS OF THE HEART AND PULMONARY ARTERY *

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Primary myxomas, fibromyxomas and fibromyxosarcomas of the heart have been recognized for many years, but they are still very rare. According to Diebold,¹ the first case was reported by von Albers in 1835, a citation which we were unable to verify. In the postmortem service of the Allegheny General Hospital we recently had a case of primary fibromyxosarcoma originating in the first portion of the pulmonary artery, extending along the pulmonary tree and producing multiple metastases in the left lung and bronchi and in the vessels of the right lung. We believe the case to be of general interest not only because it is unusual, but because the site of origin and the manner of extension appear to throw some light on the histogenesis of tumors of this type.

Fibromyxosarcomas of the heart are far less common than the myxomas from which they apparently develop. The literature dealing with cardiac myxomas contains numerous discussions concerning their true nature.

Thorel, in a series of articles on tumors of the heart published in 1903,² 1907,³ 1910⁴ and 1915,⁵ assembled reports of 24 cases that had been reported as fibromyxomas of the heart chambers. He maintained that they were organized thrombi and stated in his final report that, with the single exception of the case of Horneffer and Gautier,⁶ which had metastasized, all of the tumors were explainable on the basis of the organization of a thrombus. Among the cases cited by Thorel was that of Kesserling,⁷ who advocated the theory that fibromyxomas of the heart valves arose in embryonic rests associated with the anlagen of the heart valves, admitted to be of myxomatous tissue, and that of Bacmeister,⁸

* Received for publication August 1, 1940.

Clinical History

The patient,* A. K., was a white housewife, age 51.

Eight months before admission to the hospital the patient noticed pain about the heart and shortness of breath. She was placed at rest in bed with a diagnosis of pleuritis and, after 1 month, was apparently cured. Later the shortness of breath recurred and gradually increased. About 2 months before admission the patient suffered an attack of hemoptysis, which was not repeated. There were several gastro-intestinal upsets not accompanied by bloody stools. The patient had lost weight, although she was still rather stout. Her past history, as well as the family history, was irrelevant.

Physical Examination. The patient was well nourished and well developed. She exhibited severe respiratory distress. The skin was of normal color, and there were no palpable nodes. Eyes, ears, nose and mouth appeared normal. The chest was symmetrical, although there was limited motion on the left side, especially in the upper portion. Breath sounds, vocal fremitus and tactile fremitus were decreased over the upper, anterior portion of the chest, and no râles or rhonchi were present. There was a healed cholecystectomy scar in the right upper quadrant. Temperature, 100° F.; pulse, 98; respirations, 24; blood pressure, 110/72 on admission.

Laboratory Examinations. Laboratory findings showed the urine negative; a slight anemia; r. b. c., 4,040,000; w. b. c., 11,500; Hb., 69 per cent. Differential counts gave an excess of polymorphonuclear leukocytes up to 93 per cent. An autopsy was performed on December 2, 1939, 4 hours after death.

Roentgenologic Report. The first films were made on March 17, 1939. At that time there was a rather dense shadow at the base of the left lung and evidence of an enlarged gland in the root of the left lung. Films, made on August 9, revealed cloudiness in the lower two thirds of the left lung. There were three large tumor shadows. Those made September 26 showed that the tumor shadows had enlarged so that they were probably twice the size found at the preceding examination. On October 3 a probable diagnosis of carcinoma of the left lung was made and the patient referred for X-ray treatment. The Department of Roentgenology considered the case one of Hodgkin's disease and treated the patient on that basis. There was rapid symptomatic improvement but films made on October 19 failed to show any decrease in the size of the tumor shadows. If anything, they were slightly larger. Treatment was continued until November 8, during which time 6090 r. were given, with about 2000 r. over each of the three shadows. Following the treatment on November 8, the chest filled rapidly with fluid. On November 10 a part of the fluid was removed and air was introduced. The tumor shadows at that time had not changed in size or appearance.

Synopsis of Postmortem Findings

The body was that of a middle-aged, white female with extensive edema of the subcutaneous tissues of the chest and of both ankles.

The left pleural cavity contained about 1000 cc. of clear, straw-

* On the service of Dr. W. B. Ray and referred by Dr. William Marshall, of Aspinwall, Pa.

the data were complete, 25 were males, 9 were females and all were between the ages of 20 and 70 years. The primaries occurred in the right auricle 14 times; right ventricle, 3; pulmonary artery, 1; left auricle, 8; left ventricle, 1; auricular septum, 1; and ventricular septum, 1. Twelve cases produced metastases which occurred in the lungs 6 times; kidney, 3; lymph nodes, 3; and once each in the heart itself, pericardium, pleura, liver, pancreas and brain. The microscopic diagnoses included 15 spindle cell sarcomas, 12 round cell sarcomas, 4 giant cell sarcomas, 4 myosarcomas and 3 mixed cell sarcomas. Diebold's own case was diagnosed a "mixed cell" sarcoma, but both his description and his pictures resemble our own case closely and we believe it could have been interpreted as a fibromyxosarcoma. The only case beside our own in which the primary site was given in the pulmonary artery was that of Esbach, quoted by Diebold.

Since Diebold's ¹ report we have found 2 additional cases with metastases. Müller ¹⁸ in 1932 described a case of primary "fibrosarcoma" of the left auricular wall with secondary nodules of the lungs and pleura. Both his pictures and his microscopic description lead us to believe that his neoplasm was a fibromyxosarcoma similar to ours. Fenster ¹⁹ in 1933 reported a "malignant myxoma" of the right auricle with multiple metastases to the liver, retroperitoneal lymph nodes, kidneys, adrenals and brain.

Yater ²⁰ in 1931, in a review of all tumors of the heart and pericardium, found a total of 49 primary sarcomas of the heart. Of these, 15 had produced metastases and the diagnosis of myxosarcoma had been made in only 3 instances. Pollia and Gogol ²¹ in 1936 reviewed the incidence of heart tumors in 46,072 autopsies and found 154 primary and 220 secondary neoplasms.

As far as we have been able to ascertain, our case is the second fibromyxosarcoma occurring in the pulmonary artery.

REPORT OF CASE

Diagnoses. Primary fibromyxosarcoma at root of pulmonary artery, extensive metastases to left lung and to pulmonary arteries of right lung, pulmonary arteritis, multiple hemorrhagic infarcts of right lung, hydrothorax, edema of ankles, benign leiomyoma of uterus, and thrombosis of uterine venous plexus.

there was a second smaller nodule, not more than 3 mm. in diameter, which was red in color.

Aorta. The aorta showed slight yellowish streaking. Small atheromatous plaques and ulcerations were present in the thoracic and abdominal portions. None was calcified.

Left Lung. The left lung weighed 750 gm. and measured 20 by 11 by 6 cm. The pleura was covered with broken adhesive bands. When placed in water the lung sank except for a portion of the lower lobe. A soft, tenacious, gelatinous, tan-colored polyp protruded from the main bronchus. On section the central portion of the lung was replaced by new growths made up of nodular, gelatinous masses. At least two thirds of the lung was involved. The nodules varied from 1 to 8 cm. in diameter. Some were cystic and others contained hemorrhagic zones. Some were located in veins which they distended, some were in bronchi and others were nonencapsulated and grew freely in the parenchyma. The intravascular extensions grew as elongated polyps or resembled obturating thrombi undergoing white softening. Beneath the pleura the remaining lung was in part air-containing and in part collapsed from pressure of the tumor and the pleural effusion. Moderate anthracotic pigmentation was present.

Right Lung. The right lung weighed 450 gm. and measured 22 by 14 by 4 cm. Most of the lung was air-containing. Several of the smaller pulmonary arteries were filled with gelatinous growths. Near the apex there was a recent wedge-shaped, hemorrhagic infarct. In the lower lobe there was a grayish brown, infarcted area undergoing organization.

Uterus. The only abdominal organ that presented a gross pathologic lesion was the uterus. It was somewhat enlarged and the cavity was distorted by the presence of a fibroid tumor, 6 cm. in diameter, located in the posterior wall. At the margin of the growth there were several dilated veins of the uterine plexus, filled with thrombi. Most of these were deep red in color, although some appeared to be well organized and of a red-brown color.

Microscopic Examination

Heart. The cardiac muscle cells were of normal size and staining qualities.

colored fluid and the right about 500 cc. The left lung was attached by dense, fibrous bands covering all of the pleural surfaces except the diaphragmatic. The right lung was adherent at the apex and over the posterior portion of the heart.

Heart. The pericardial sac contained 200 cc. of clear fluid. *In situ* the heart was distended. The right auricle was greatly dilated. The apex was situated beneath the fifth interspace in the axillary line and the base extended to the right beyond the sternal margin. When empty the heart weighed 400 gm. and measured 14 by 11 by 5 cm. The epicardium was smooth and glistening. The subepicardial fat was excessive, and watery in appearance. The myocardium was flabby and dark red. The auricular and ventricular cavities were normal. The pulmonary valve orifice was enlarged and the pulmonary artery was increased in diameter. All other valvular orifices were of average size and all valve cusps were pliable and apparently in good condition. The left ventricular wall was 1.3 cm. in thickness.

In the pulmonary artery three myxomatous polyps were attached to the posterior wall at a point about 3 cm. above the corpus arantii of the middle semilunar cusp. The polyps hung downward in the pulmonary artery and almost occluded it. They were grayish in color and were of aspic-gelatin appearance and consistency. Their pedicles were united in a common, broad base. The largest polyp was roughly pear shaped and measured 3 cm. in length and 2.5 cm. in its greatest diameter. It was pendulous and hung freely over the other two polyps and slightly to the right of the next smaller one. This one was egg shaped and had a very short pedicle. It measured about 2.5 cm. in all diameters. The smallest polyp measured 1.5 cm. in length by 0.6 cm. in diameter and was completely obscured until the largest polyp was lifted. The surfaces of all the polyps were smooth, glistening and semitransparent. For at least 2 cm. to the left, 1 cm. above and below and 3 cm. to the right of the base of the polyp the arterial wall was thickened, reddened, appeared ulcerated and was covered with a layer of fibrin of varying thickness. Directly over the center of the right semilunar cusp there was a thickened nodule about 8 mm. in diameter which protruded for a distance of 4 mm. into the lumen. It was hemorrhagic but appeared to be solid throughout. About 1 cm. above the right semilunar valve

ary artery except that it was unattached at the point where the section had been taken. Other sections containing large branches of the pulmonary artery showed them to be filled with neoplastic thrombi. Some of these completely closed the vessel and infiltrated the walls, while others were mural with finger-like polypi extending out of the main masses. One vessel showed several points at which the tumor was penetrating its walls and invading the lung. At the points of perforation the cells were growing at right angles to the vessel wall. The secondary nodules in the lung tissue showed concentration of the cellular and fibrous elements in their peripheries but appeared to displace and compress the surrounding lung tissue rather than to form definite capsules. In the right lung there were two areas of hemorrhagic infarction, one of which was partially organized. Vessels leading into these areas were filled with simple emboli without tumor cells. It was thought that these originated from the thrombi in the uterine plexus.

Uterus. The tumor of the uterus was a very dense leiomyoma of the fibroid type in which multiple sections failed to show any unusual growth activity.

DISCUSSION

The neoplasm in this case was a malignant tumor originating at the root of the pulmonary artery and belonging in the category of myxomas, fibromyxomas and fibromyxosarcomas of the endocardium.

Microscopically it could have been called a spindle cell sarcoma, a mixed cell sarcoma or even a giant cell sarcoma if cellular morphology alone had been considered. The diagnosis of fibromyxosarcoma was made on the presence of large, spongy, round and spindle-shaped cells with stellate fibrils spreading in the loose meshes of the stroma, and on the nature of the stroma itself. The cells varied greatly in size and in the number of nuclei. Many were in mitosis. The stroma was typically myxomatous with peripheral zones of collagenous fibrils. It was watery in appearance and tended to undergo necrosis and form cysts. The type of stroma offered no support to the proliferating capillaries and areas of hemorrhage were common. The metastatic growths established the diagnosis of sarcoma.

As to the origin of polypoid tumors of the heart, the views of

Polyps of Pulmonary Artery. A striking feature about the polyps was the great excess of myxomatous stroma in comparison to the cellular elements. The external surfaces were smooth and probably had been covered with endothelium. There was a concentration of the stroma just beneath the surface and this zone was more cellular than the central part of the polyp. At the junction of the polyp with the pulmonary arterial wall there was an interchange of cells and vessels with those of the pulmonary artery. The most cellular part of the growth was near the attachment. Here the cells were very numerous and varied greatly in size and shape. Some of the cells were spindle shaped with long, stellate processes, and others were round or oval. Many of the cells were multinucleated and appeared to be true neoplastic forms. The multinucleated cells had from 2 to 30 or more nuclei. Some of them resembled striped muscle cells but could not be shown by special methods to have cross striae. In addition to the tumor cells there were numerous single and multinucleated phagocytes, occasional lymphocytes and numerous red cells. Mitoses were numerous in some zones. In the more cellular zones there was a fair amount of collagen in the stroma. As the central portions of the polyp were approached there were fewer tumor cells and many of those that were present were unstained. Near the central portion the stroma was very edematous; large, cystic spaces were present, and numerous hemorrhagic areas were seen. The number of vessels varied and most of them were capillaries or thin-walled arterioles. The sessile nodule of the pulmonary artery proved to be a deep-seated, hemorrhagic, atheromatous cyst.

Pulmonary Artery Near Attachment of Polyp. The intima was ulcerated and covered with adherent, hyalinized fibrin undergoing organization. This zone contained many mononuclear phagocytes and less numerous multinucleated giant cells. Beneath the ulcerated surface the intima and part of the media presented a myxomatous appearance and were infiltrated with typical neoplastic cells of the type already described.

Aorta. The aorta showed a moderate degree of intimal sclerosis.

Lungs. A section of the main bronchus showed a very active inflammatory process. The lumen was filled with a myxomatous polyp of exactly the same structure as that found in the pulmon-

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Thorel, Czapek and others that they are organized thrombi could be excluded at once in our case. The location of the tumors on the wall of the pulmonary artery at some distance from any point at which myxomatous anlagen are known to exist makes it extremely unlikely that they arose in an embryonic rest. The interpretation of Bacmeister and others that these tumors are derived by myxomatous metaplasia within a thrombus could not be excluded. In view of the facts that there was an ulcerated area of the pulmonary arterial wall covered by a hyaline, mural thrombus in the immediate vicinity of the pedicle, that there was no line of separation between the arterial wall and the elements of the tumors, that the blood supply of the new growth was continuous with vasa vasorum and that fibroblasts, tumor cells and capillary endothelium were common to the growth and to the arterial wall and were generously intermingled, we feel that organization and metaplasia offer the most logical explanation. It is no more difficult to accept the possibility of neoplastic metaplasia in a zone undergoing reparative proliferation than it is to accept the abrupt establishment of a malignant growth in otherwise apparently normal tissues.

CONCLUSIONS

1. An example of fibromyxosarcoma of the pulmonary artery is described. This is probably the second tumor of its type to be reported in this location.

2. The primary growths resembled organized thrombi in the gross, yet presented all of the microscopic evidences of malignancy.

3. There were extensive metastases along the pulmonary arterial tree and in the lungs and bronchi.

4. There was a zone of pulmonary arteritis, about the pedicle of the tumor, covered with an organizing thrombus that showed no neoplastic changes.

5. The association of pulmonary arteritis with the base of the tumor suggests that a logical sequence in the tumor's development may have been: pulmonary arteritis with loss of endothelium, protective thrombosis, organization of the thrombus with myxomatous metaplasia, sarcomatous change in the myxoma and secondary extensions to the lungs by way of the branches of the pulmonary artery.

DESCRIPTION OF PLATES

PLATE 51

FIG. 1. Gross photograph of the heart showing the attachment of one small and two large polyps to the wall of the pulmonary artery 3 cm. above the middle semilunar cusp. Note the thickening and roughening of the pulmonary artery.

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PLATE 52

FIG. 2. Gross photograph of left lung showing extensive secondary myxomatous tumors. In the center a polypoid mass may be seen protruding from the main bronchus. The large, bulbous structure in the lower right-hand corner was a cystic, polypoid mass which was squeezed out of the lung when it was sectioned.



1

PLATE 53

FIG. 3. Low power photomicrograph through the smallest of the three polyps and showing the continuity of the growth with the pulmonary arterial wall. The cellular areas of the tumor were just beneath the surface and along the line of attachment. $\times 22$.

FIG. 4. Wall of the pulmonary artery at a point near the attachment of the polyp. Note the arteritis, ulceration and deposit of hyaline fibrin on the surface. $\times 30$.



2

PLATE 54

FIG. 5. Section of the pulmonary artery containing a secondary myxomatous growth. The vessel is, for the most part, occluded, although parts of the tumor are growing as long, filamentous polyps. $\times 8.5$.

FIG. 6. A small branch of a pulmonary artery, the wall of which was penetrated at several points by direct extensions of the tumor into the parenchyma of the lung. $\times 30$.

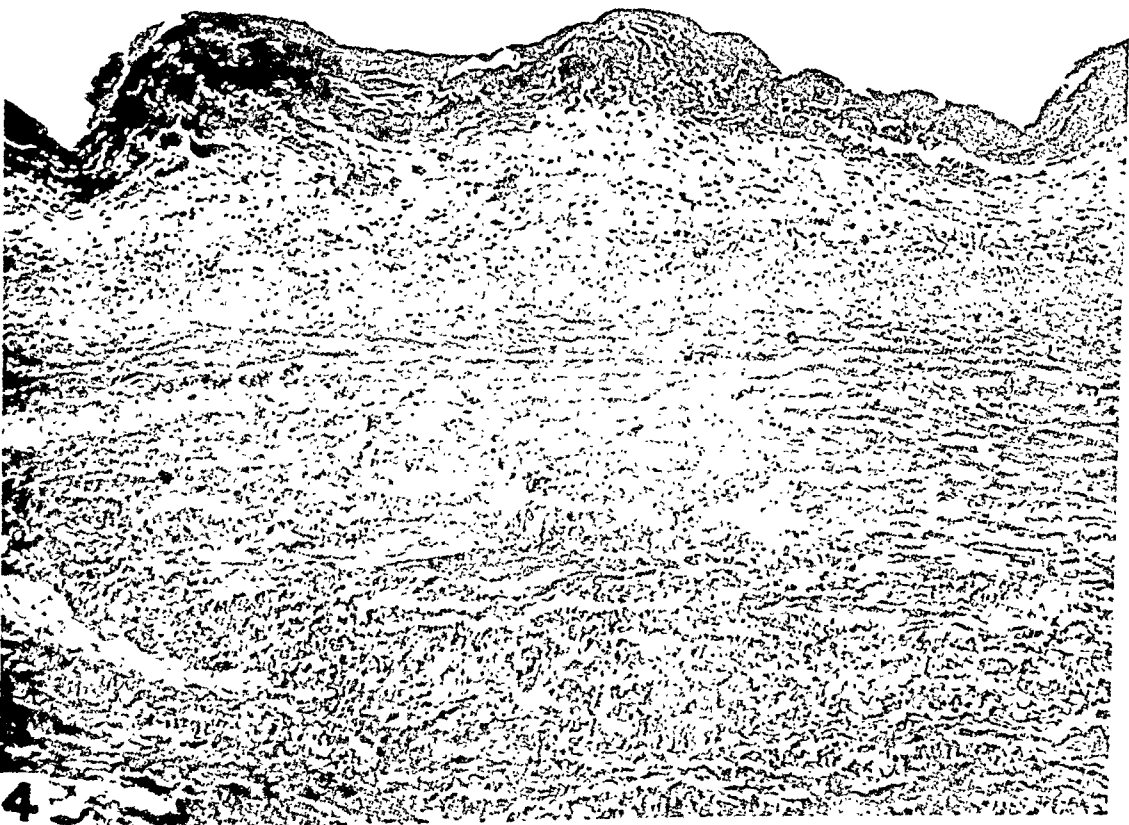
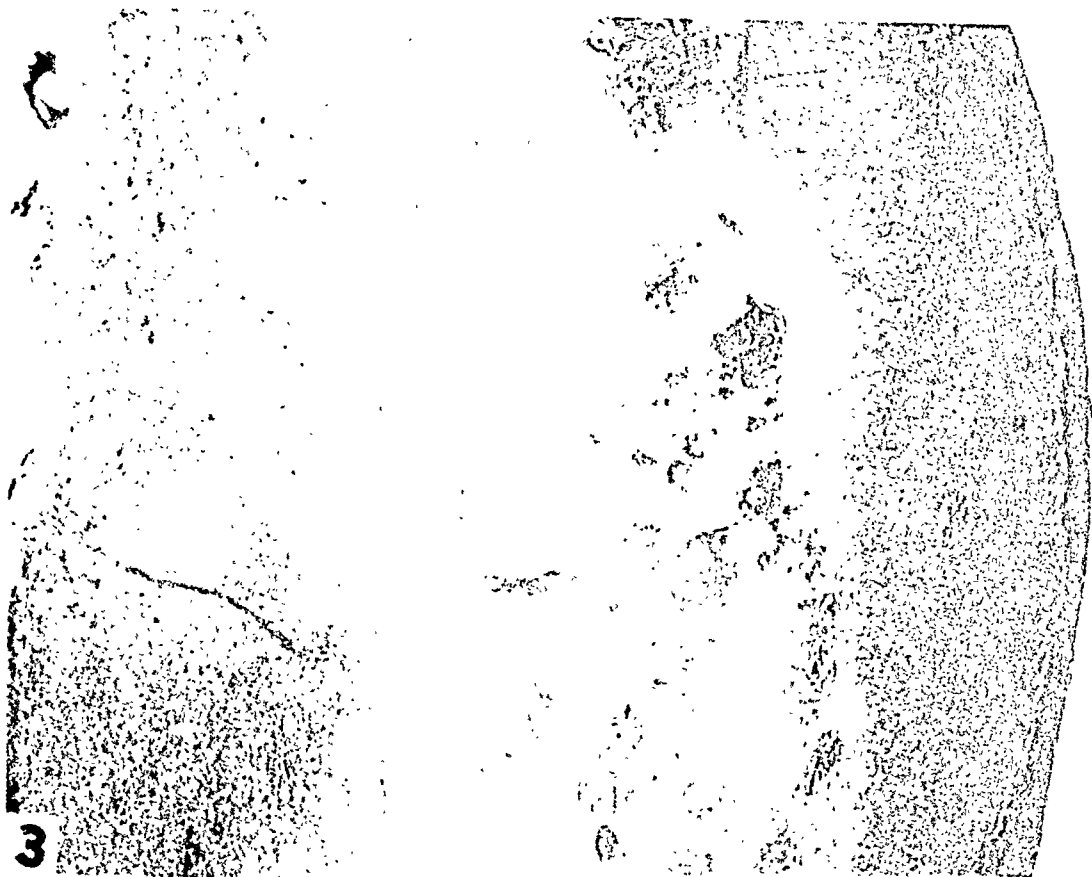


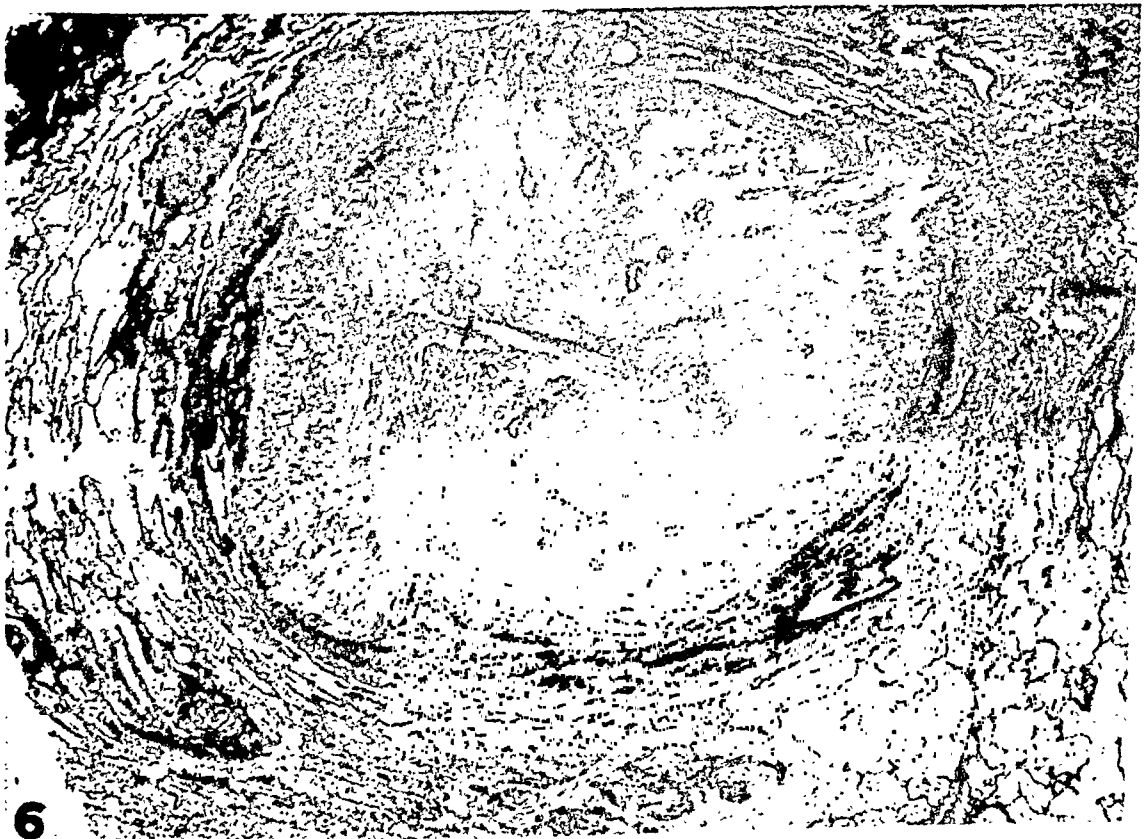
PLATE 55

FIG. 7. High power photomicrograph of one of the secondary tumors of the lung, showing a great variation in the shape and size of the tumor cells and many multinucleated forms. $\times 180$.

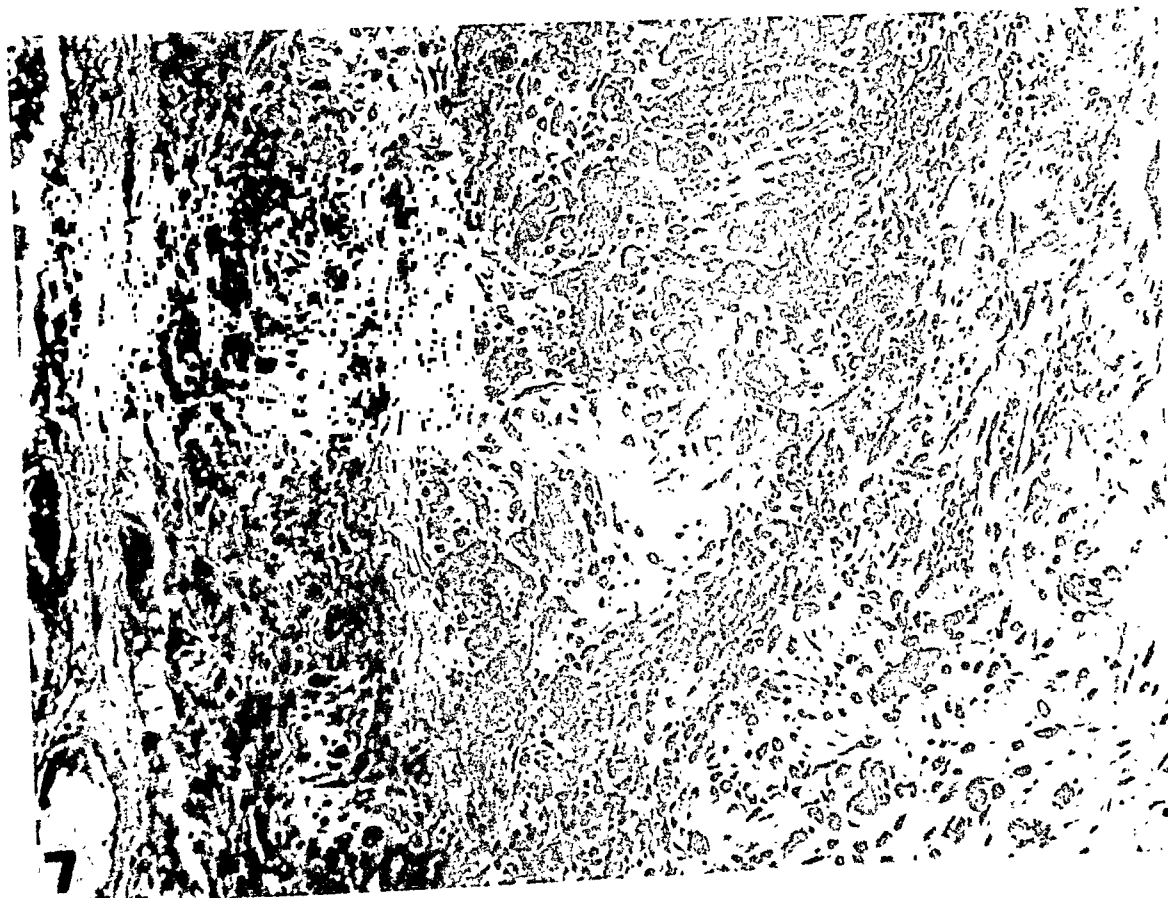
FIG. 8. High power photomicrograph of a secondary polyp extending through the wall and growing freely in a small bronchiole. $\times 180$.



5



6



Fibromyxosarcomas of the Heart

into Bowman's space had occurred were microorganisms cultured from the urine.

We decided to perform experiments, using particularly the method employed by Book,¹¹ attempting to determine to what extent normal kidneys can excrete living bacteria, in what ways the kidney can remove living bacteria and other particulate materials from the circulating blood, and the localizing potentialities of the kidney for such particles, both in normal organs and in those in which acute experimental hydronephrosis had been produced.

MATERIALS AND METHODS

Adult male rabbits were used. The problem of excretion of living bacteria into the urine was studied by injecting 24-hour broth cultures of *Staphylococcus aureus*, *Bacterium coli* or *Bacillus typhosus* intravenously into the rabbits. These animals had previously been anesthetized with nembutal and their bladders exposed by low laparotomy. At intervals of from 5 to 120 minutes, samples of urine were withdrawn from the bladder with a sterile syringe after searing the surface of the bladder with a red-hot spatula. Duplicate samples of 1 cc. each were mixed well with warm, melted agar culture medium and incubated at 37° C. for 48 hours.

The second procedure was to determine the influence of experimental hydronephrosis upon bacterial localization in the kidneys. Hydronephrosis was produced in the left kidney of each rabbit by ligating the ureter after either a mesial incision and transperitoneal approach or a left flank incision and extraperitoneal approach. In some rabbits we produced only a partial obstruction by placing an 18 gauge hypodermic needle under the ligature, with removal of the needle after the ligature had been tied. The injections were made 72 hours later, at which time acute hydronephrosis was well developed.

The influence of such hydronephrosis upon the ability of the kidney to remove particulate materials from the blood was studied in two ways. (1) Rabbits were injected intravenously with India ink (Higgins's White Label or Pelican) diluted 1:4, 1:10, or 1:20 with sterile normal salt solution. Seventeen animals were killed at varying periods after injection and liver, spleen and both kid-

BACTERIAL LOCALIZATION IN THE KIDNEYS *

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Evidence as to the rôle of the kidneys in the removal of bacteria from the blood and their excretion into the urine is conflicting. Although microorganisms not infrequently enter the blood stream and pass through the vascular bed of the kidneys, there is still uncertainty as to the extent to which normal kidneys can remove them from the blood and destroy them or excrete them through the urine. Some workers have concluded that normal kidneys can excrete bacteria (Biedl and Kraus,¹ Schweizer,² Rolly,³ MacKenzie and Cochrane⁴ and Cohnheim⁵) while others have come to opposite conclusions (Pernice and Scagliosi,⁶ Sherrington,⁷ Asch,⁸ Matsuyama,⁹ Dyke,¹⁰ Book,¹¹ Helmholtz and Millikin¹² and Kirkpatrick¹³).

These conflicting opinions are probably due to differences in experimental procedure depending upon the number of bacteria injected, conditions and time intervals under which the urine was collected and cultured, and the presence of unsuspected renal lesions. They might also be due to the production of mechanical or other injury to the kidneys during the course of the experiments. In some instances in which bacteria were injected intravenously or into the renal artery, glomerular lesions could be demonstrated. Book,¹¹ for example, injected living staphylococci intravenously into rabbits and was unable to find them in the urine until about 12 hours later. Large numbers, in fact, did not appear until after 18 to 24 hours. When he injected colon bacilli, the organisms were not found in the urine until 48 hours had elapsed. Histological studies, made by removing the kidneys and incubating them at 37° C. in order to allow the bacteria in the kidneys at the time of removal to develop into colonies, showed the bacteria, in most instances, in capillaries of the cortex and medulla. Only in animals in which growth into the glomeruli and

* Received for publication July 18, 1940.

TABLE I
Excretion of Bacteria in the Urine after Intravenous
Injection into Normal Rabbits

Animal No.	Organism	Time elapsed	Urine culture (2 plates)	
		minutes		
1.	<i>Staph. aureus</i>	5	no growth	no growth
2.	<i>Bact. coli</i>	5	no growth	contam. staph.
		15	no growth	no growth
3.	<i>Bact. coli</i>	15	no growth	contam. <i>B. subtilis</i>
4.	<i>B. typhosus</i>	5	no growth	no growth
		20	no growth	no growth
5.	<i>B. typhosus</i>	8	no growth	no growth
		30	no growth	no growth
6.	<i>B. typhosus</i>	5	no growth	no growth
		20	no growth	no growth
		50	no growth	no growth
		70	contam. <i>B.</i> <i>subtilis</i>	no growth
		120	no growth	no growth

TABLE II
Localization of India Ink in Normal and Hydronephrotic
Kidneys after Intravenous Injection

Injection	Animal No.	Time	Normal	Hydronephrotic
10 cc. of 1:4 ink	3	3 min.	+	+++
	4	7 min.	+	++
	20*	3 hrs.	+	++++
	18*	23 hrs.	trace	+++
	16	24 hrs.	+	++
	26*	25 hrs.	trace	++
20 to 40 cc. of 1:4 ink	22	4 hrs.	++++	++++
	23	5 hrs.	++++	++++
	24*	5 hrs.	+++	++++
	21†	25 hrs.	+++	++++
20 to 40 cc. of 1:10 ink	28*†	12 hrs.	+	++
	7	22 hrs.	+	++
	27*†	24 hrs.	trace	+++
	25	24 hrs.	+	++
20 cc. of 1:20 ink	6*	14 hrs.	trace	+
	5	24 hrs.	trace	trace
20 cc. of 1:40 ink	29*†	12 hrs.	+++	+++

* Intracellular carbon was seen in the hydronephrotic kidneys. This, in most cases, was in large phagocytic cells in the areolar tissue of the pelvis.

† The right kidney in this rabbit displayed both gross and microscopic evidence of a previous inflammatory process. Both extracellular and intracellular carbon particles were seen in large amounts.

‡ These animals received intravenous injections of Pelican ink. The others received Higgins's White Label.

neys were removed. Large transverse sections from the kidneys and also other tissues were stained with hematoxylin and eosin. (2) A 24-hour broth culture of either *Staph. aureus* or *Bact. coli* in 1 or 2 cc. portions was injected intravenously. At various intervals the rabbits were sacrificed. The kidneys were removed aseptically, placed in sterile Petri dishes and incubated at 37° C. for 24 hours in order to allow any bacteria present at the time of removal to develop colonies. After incubation the organs were fixed in Zenker-formaldehyde solution and microscopical sections stained by the Claudius modification of Gram's method.

By these methods one can compare the two kidneys exposed simultaneously to particulate materials in the blood stream and can observe the extent to which the mechanical effects of increased intrapelvic pressure may modify the blood flow and thus influence bacterial localization in the hydronephrotic kidney. Sections reveal where the particles are deposited in the kidney and thus help to disclose the mechanism influencing their deposition.

RESULTS

Experiment I. The Excretion of Bacteria into the Urine after Intravenous Injection

Six rabbits were injected intravenously: 1 with *Staph. aureus*; 2 with *Bact. coli*; and 3 with *B. typhosus*. The results showed that in no instance did any of the injected bacteria appear in the agar plates from the urine samples, indicating that under these conditions the normal kidneys did not excrete bacteria in significant numbers during the period of the experiments (Table I).

Experiment II. The Effects of Experimental Hydronephrosis upon the Removal of Particulate Materials by the Kidney

Our first studies in this experiment were concerned with the distribution of carbon particles in the kidneys of rabbits with a left hydronephrosis of 72 hours' duration.

On gross examination at autopsy, performed from 3 minutes to 25 hours after injection, the black staining in the spleen, omentum and, in some cases, the liver and lungs was intense. The hydronephrotic kidneys in all cases weighed about twice as much as the normal kidneys. The pelves were dilated in all cases and each contained about 3 cc. of urine. This urine, in a few in-

few cases appeared to be purulent. It was not cultured. No gross abscesses were seen in kidneys or other viscera.

A quantitative estimation of the influence of hydronephrosis on the removal by the kidneys of living bacteria injected intravenously was difficult to make. We studied transverse sections of the kidneys taken in the midportion in order to get fairly uniform sections which would include all elements of the renal structure. However, the bacterial colonies were not evenly distributed. Therefore we could not count bacterial colonies per field, but we estimated the number of colonies in the sections studied.

The bacterial colonies appeared as blue-staining masses whose shape was determined to some extent by the portions of the kidneys in which they were located. Colonies were seen in both normal and hydronephrotic kidneys, but were much more numerous in the latter. Their usual location was in the pelvic region, where they were commonly circular in section. It was, however, difficult to determine the exact anatomical location of these colonies because in growing they exerted a distorting force on surrounding structures and destroyed anatomical relationships.

In the cortex, colonies developed in the glomerular capillaries, where they grew as rounded colonies rather than in the crescentic shape that they would have to assume to fit between the glomerular tuft and the capsule.

Colonies were also seen interstitially in the medulla. Here, it was often difficult to determine whether they were located in tubules, blood vessels or lymphatics because the majority of them had assumed an elongated form running parallel to the tubules.

The results following injections of different bacteria were qualitatively similar. Those animals injected with *Bact. coli* showed slightly more colonies per section, although the location of the colonies was the same for *Bact. coli* and *B. typhosus*.

In an effort to approach more closely the conditions which might exist in clinical cases of ureteral occlusion, partial occlusion was produced in several rabbits by the method described. The results in these animals did not differ from those obtained after complete ureteral obstruction.

In several rabbits that had received intravenous injections of living bacteria, the liver and spleen also were examined by the in-

stances, was stained by the India ink, especially in those animals that had received relatively large amounts of ink. On cut section one could distinguish a grayish cast to the renal tissue, especially of the hydronephrotic kidneys. Otherwise there was little difference in the gross appearance of the normal and hydronephrotic kidneys.

The results of the histological examination of the kidneys of these rabbits injected with India ink are tabulated in Table II. In general, most of the carbon particles were present in masses in the glomerular capillaries, in greater amounts in the hydronephrotic than in the normal kidneys. The amount of carbon in a given section is expressed in terms of from 1 plus to 4 plus, depending upon the number of carbon particles per low power field. In most instances, some of the carbon particles had passed through the glomerular capillaries and had accumulated in the intertubular blood vessels. In the medulla, in addition to the carbon in the blood vessels, small granules or clumps were seen in the tissue spaces between dilated tubules. Although particles were seen in this location in both normal and hydronephrotic kidneys, they were present in much greater numbers in the latter. In the pelves of the hydronephrotic kidneys, carbon particles frequently lodged either singly or in clumps just beneath the pelvic epithelium in the areolar tissue. This was not so common or so marked in the normal kidneys.

In several rabbits carbon particles were seen within fixed phagocytic cells of the peripelvic areolar tissue, especially beneath the epithelium. In 2 rabbits carbon particles were present within large mononuclear cells located interstitially in the medulla. These intracellular carbon particles were seen only in the hydronephrotic kidneys. In 1 animal (No. 21) the right kidney, both grossly and microscopically, showed definite signs of a previous inflammatory process and in it there was much more carbon, both intracellularly and extracellularly, than in any of the other animals.

The gross appearance of the kidneys and other viscera of 29 rabbits that had received intravenous injections of bacterial cultures was not striking. The hydronephrotic kidneys again were found to weigh about twice as much as the normal kidneys. The dilated pelves contained an average of 3 cc. of urine, which in a

sumably because the quantities of carbon which we used were too small to obtain this effect, and also because the endothelium of the blood vessels does not normally have any marked phagocytic activity.

From our data, and from a review of the literature, we feel certain that normal kidneys do not possess a marked tendency to localize or to excrete blood-borne bacteria or other particulate matter. When the ureter is occluded, however, or when overwhelming doses of particularly virulent bacteria reach the kidneys, pyelonephritis may occur. Clinically, there is frequently demonstrable obstruction of the ureters in cases of pyelonephritis, as has been pointed out by Lieberthal,¹⁹ Weiss and Parker²⁰ and others. This finding has been borne out experimentally by Brewer,²¹ Lepper,²² and more recently by Mallory, Crane and Edwards.²³ Lepper has stressed the point that ureteral obstruction of even short duration may be sufficient to favor bacterial localization in the kidney. We feel that our findings confirm those of these authors. The sequence of events in the production of acute pyelonephritis has been well described by Mallory. We have shown how the bacteria lodge in these hydronephrotic kidneys as the first stage in the process leading to acute pyelonephritis.

CONCLUSIONS

1. The normal kidney usually does not excrete living bacteria circulating in the blood.
2. Blood-borne foreign particles have but a slight tendency to localize in the kidneys. Localization, when it occurs, is due to the narrow caliber of the glomerular and medullary capillaries, which tend to trap large or clumped particles.
3. Acute experimental hydronephrosis increases the tendency for blood-borne particulate matter to localize in the kidney.
4. This localization results from stasis, reinforced by the sterile inflammatory process and the mobilization of phagocytic cells.

NOTE: We wish to thank Paul R. Cannon for valuable assistance and advice during the course of this work.

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cubation and Gram staining procedure described. As might be expected, numerous colonies were demonstrated in both organs.

DISCUSSION

It is now well known that localization of intravenously injected particulate matter occurs principally in those organs containing large numbers of phagocytic cells and a sinusoidal type of blood flow, whereas but little localization occurs in organs poorly supplied with macrophages and with a rapid flow of blood through vessels lined with common endothelium (Sullivan, Neckermann and Cannon¹⁴). For this reason, bacteria in the blood stream usually do not localize in the kidneys. It is not surprising, however, that at times larger particles, such as carbon, may lodge to some extent in the narrow lumen of the glomerular tuft or, if this is passed, in the smaller capillaries of the medulla.

In hydronephrotic kidneys, on the other hand, certain conditions tend to favor a localization of blood-borne particulate matter. Lucas¹⁵ has shown that an increase in intrapelvic pressure slows the circulation of blood through the kidney and produces some renal damage, as evidenced by the finding of blood in the urine in the hydronephrotic sac. Helmholtz and Field¹⁶ have demonstrated that sterile inflammation occurs in the renal parenchyma and the pelvis after ligation of the ureter and that phagocytic cells become more numerous in the peripelvic areolar tissues and in the interstitial tissue of the involved kidney.

Other explanations have been advanced to account for the localization of blood-borne particulate matter in the kidneys. MacKenzie and Hawthorne¹⁷ have demonstrated that carbon particles will remain in the renal substance for long periods after intravenous injection. Helmholtz and Millikin¹² concluded that carbon particles may be found within the endothelial cells of the renal capillaries. Brickner¹⁸ reported finding carbon particles within endothelial cells of the blood vessels after repeated heavy doses of carbon and attributed this to: (1) a "blockade" or saturation of the reticulo-endothelial system; (2) the tortuosity of the renal capillaries leading to a considerable stasis of blood flow; and (3) the smaller diameter of the capillaries furthering the approximation of particles to endothelium. We did not find carbon particles in endothelial cells of any of the material studied, pre-

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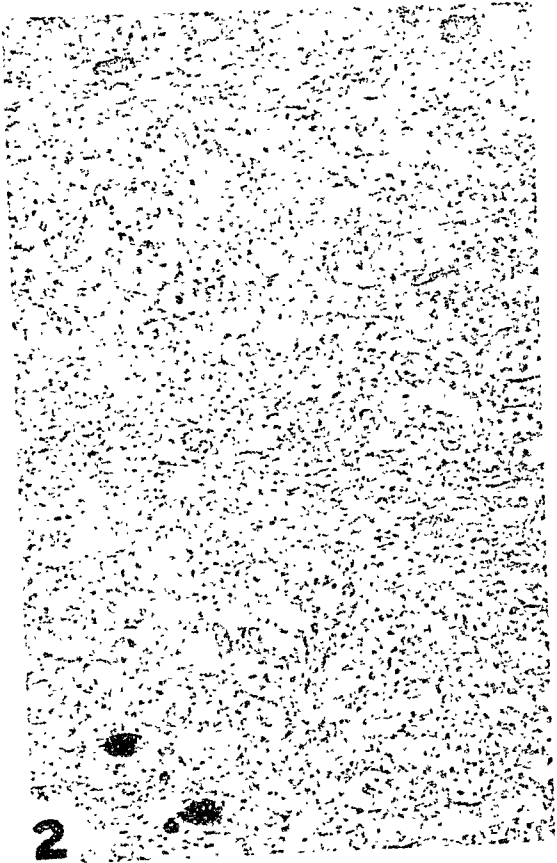
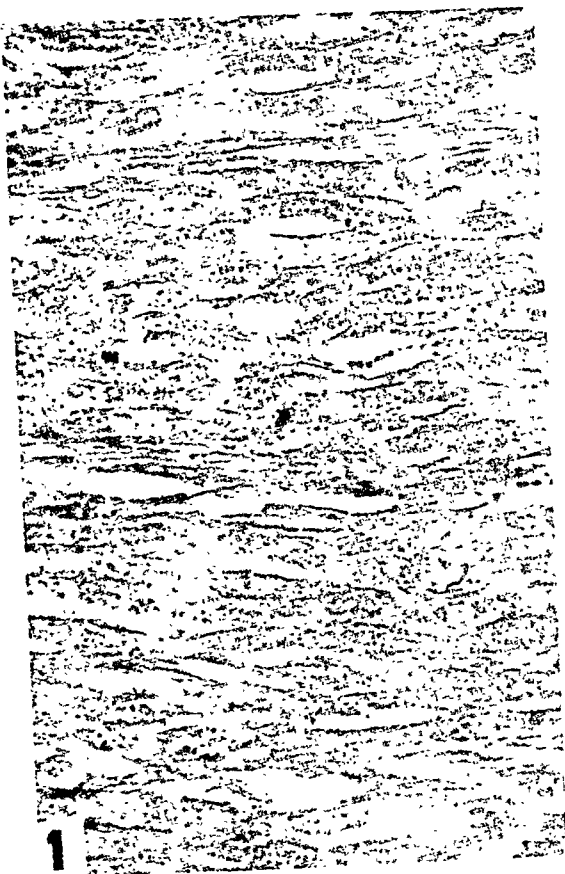
DESCRIPTION OF PLATE

PLATE 56

The kidneys from which these photomicrographs were made had been incubated for 24 hours before fixation, with resulting loss of cellular detail through autolysis.

- FIG. 1. Rabbit No. 9. This animal received 1 cc. of a 24-hour broth culture of *Staphylococcus aureus* intravenously and was sacrificed 2 hours later. This is a section of the normal kidney showing a few bacterial colonies which are quite small. They appear to be lodged between the tubules, probably in capillaries. $\times 125$.
- FIG. 2. Rabbit No. 9. Photomicrograph of a hydronephrotic kidney. The dark-staining areas are bacterial colonies. The elongated colonies seem to lie between the tubules, whereas the rounded ones are probably within blood vessels or are lying free in the tissue spaces. $\times 125$.
- FIG. 3. Rabbit No. 10. This animal was killed 4 hours after intravenous injection of 1 cc. of a broth culture of *Staphylococcus aureus*. Section of the normal kidney shows a few pin point bacterial colonies. $\times 125$.
- FIG. 4. Rabbit No. 10. A hydronephrotic kidney which contains several large bacterial colonies. One of these is surrounded by tubular epithelium. The others seem to lie in blood vessels. $\times 125$.

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Bacterial Localization in Kidneys

closed the presence of metastatic hypernephroma (Pemberton and Bennett,⁵ H'Doubler⁶ and Welti and Huguenin⁷).

Our interest in this condition was aroused by noting the development of a nodule in the right lobe of the thyroid gland of a patient who had undergone a combined abdominoperineal resection 6 months previously for adenocarcinoma of the rectum, grade I+. Extirpation of the right lobe of the thyroid gland revealed two hard, white, circumscribed nodules, which on histologic examination were found to resemble closely the rectal lesion. Accordingly, we have undertaken a study of all of the metastatic tumors of the thyroid gland which could be found in the museum of the Mayo Clinic.

CLINICAL STUDY

Nineteen cases were found in which metastasis to the thyroid gland had taken place. In 3 cases, thyroidectomy had been performed and led to a correct diagnosis during the life of the patient (2 of these cases have been reported by Pemberton and Bennett⁵). There were 7 cases in which some abnormality of the thyroid gland was noted on examination of the patient, but in which its true nature was not ascertained until after death. In 9 cases, metastatic lesions in the thyroid gland were an incidental finding at postmortem examination. In addition to these cases,

TABLE I
Distribution of Cases According to Origin of Primary Growth

Site of primary lesion	Confirmed cases*	Unconfirmed cases†
Lung	4	2
Kidney	2	1
Pancreas	2	0
Male breast	2	0
Female breast	0	4
Face	2	1
Stomach	1	0
Adrenal gland	1	0
Rectum	1	0
Cervix	1	0
Parotid gland	1	0
Bladder	1	0
Lymphosarcoma	1	0
Esophagus	0	6
Melanoblastoma	0	1
Sarcoma	0	2
Totals	19	17

* By pathologic study.

† No pathologic study made.

EXOGENOUS TUMORS OF THE THYROID GLAND *

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The infrequency with which metastatic tumors are found in the thyroid gland long has attracted the attention of pathologists. This relative immunity to secondary implants, occurring in an organ whose blood supply per unit of weight exceeds that of any other in the body, has presented an interesting topic for speculation.

That this immunity may be more apparent than real has been suggested by a number of investigators, who have pointed out how seldom a careful study of the thyroid gland is made at routine postmortem examination. Willis¹ found metastatic lesions in the thyroid gland in 5.2 per cent of cases in which death was due to carcinoma. Rice² made painstaking microscopic studies of the thyroid glands in cases in which carcinoma was the cause of death and found embolic tumor cells in 8 of 89 glands examined.

Although the total number of cases with metastatic tumors of the thyroid gland which have been reported is comparatively small (less than 200), metastatic lesions have been described from tumors in almost every region of the body. There appears to be no correlation between the relative frequency of occurrence of various tumors and the incidence of metastatic lesions in the thyroid gland. Although the gastro-intestinal tract is the most common site of carcinoma, few cases have been reported in which tumors in this situation have spread to the thyroid gland. We were able to find only 3 reports in which the colon or rectum was the primary site of disease (Naegeli,³ Willis,¹ and Rankin and Fortune⁴). Carcinoma of the breast, melanoblastoma and carcinoma of the lung have been most frequently described as establishing secondary foci in the thyroid gland. In most cases, metastatic lesions in the thyroid gland have been incidental findings at necropsy, but on a number of occasions thyroidectomy has dis-

* Received for publication July 19, 1940.

In 2 there was diffuse infiltration of the stroma of the gland by the cells of squamous cell carcinomas from the bladder and lip, respectively. In the remaining 4 cases the metastatic lesions were present as macroscopically circumscribed nodules, single in the case of a mixed tumor of the parotid gland and of an oat cell carcinoma of the lung and multiple in the case of neuroblastoma of an adrenal and of a hypernephroma. These circumscribed nodules may have represented invasion of preëxisting adenomas. In 12 cases, the involved glands were frankly adenomatous in varying degrees. In 1 of these cases the gland was invaded directly by a lymphosarcoma; in another indirectly by carcinoma of the lung which had extended into the thyroid gland from an involved supraclavicular lymph node. Diffuse infiltration of the thyroid gland occurred in the case of an adenocarcinoma of the stomach, grade IV, and of a squamous cell carcinoma of the cheek. In the former, the acini themselves were invaded to an unusual degree, in contrast to the more common limited invasion of the connective tissue elements of the gland with secondary displacement and destruction of the parenchyma. In the remaining 8 cases, macroscopically circumscribed metastatic nodules were present. In 1 of these, a tiny, solitary, metastatic lesion from a squamous cell carcinoma of the bladder was noted. In the remainder (1 from an adenocarcinoma of the rectum, 2 from adenocarcinomas of the pancreas, 2 from adenocarcinomas of male breasts, 1 from an adenocarcinoma of the lung and 1 from a hypernephroma), multiple metastatic nodules were present, either isolated or gathered around the walls of adenomas.

In a number of cases, the origin of the tumor cells could be surmised from a study of the thyroid gland alone. Hypernephroma and adenocarcinoma of the bowel could be identified from their metastatic lesions. In all cases, the grade of the metastatic tumor corresponded with the grade assigned to the primary tumor. There seemed to be little possibility of confusing any of the lesions studied with primary neoplasms of the thyroid gland. Varying degrees of fibrosis, necrosis and lymphocytic infiltration were noted.

there were 17 cases in which there was clinical evidence of malignancy of the thyroid gland coexistent with or secondary to a neoplastic process elsewhere but in which no confirmatory pathologic study was carried out. There were 6 cases in which primary malignant lesions of the thyroid gland were coexistent with malignancy elsewhere.

The sites of the primary tumors in the group of 19 confirmed cases and 17 unconfirmed cases are shown in Table I. The remainder of this study is limited to a consideration of the confirmed cases.

In all but 2 cases, the metastatic lesions in the thyroid gland were part of a generalized carcinomatosis. In 1 of these, involvement of the regional lymph nodes was found at the time of operation on the primary lesion, but in the second no evidence of other metastatic lesions was found.

In 4 cases, the primary lesion had been removed surgically. In 2 cases, the examination of tissue removed by thyroidectomy was indicative of the presence of a primary lesion elsewhere. In 1 of these, the primary lesion subsequently was removed as there were no evidences of other metastatic foci.

In reviewing the cases it was found that in only one was there a history of previous disease of the thyroid gland. This patient, who had undergone thyroidectomy 12 years previously for exophthalmic goiter, returned with symptoms of recurrent hyperthyroidism and a basal metabolic rate of plus 27. The patient died from peritonitis following radium treatment of a squamous cell carcinoma of the cervix. At necropsy, the thyroid gland was found to contain metastatic carcinoma from the cervix. In 2 cases, "benign" goiter was described at the time of admission and in 4 cases, examination suggested "malignant" goiter, but in none of these cases was the ultimate pathologic diagnosis established during life. Among the 3 cases in which the diagnosis was made during life, there was a clinically "benign" goiter present in 2 and a clinically "malignant" goiter in 1.

PATHOLOGIC STUDY

In 7 cases, the implants of tumor tissue seemed to have been established in previously normal glands. In 1 of these an adenocarcinoma of the lung had gained entrance by direct invasion.

SUMMARY

A study is presented of 19 cases in which the thyroid gland was involved by a malignant tumor that arose elsewhere in the body. Metastatic tumors are found to occur more commonly in the adenomatous thyroid gland than in the normal thyroid gland.

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COMMENT

It has long been recognized that primary carcinoma of the thyroid gland customarily arises in goitrous glands. It would also appear that metastatic tumors are found with greater frequency in adenomatous glands than in normal glands. This was true in 12 of the cases in this study and possibly in 4 others out of a total of 19.

Because of the infrequency with which the thyroid gland is involved by metastatic lesions, it has been suggested that the normal thyroid gland does not afford the "congenial soil" for tumor implants of which Paget⁸ has written. Some have felt that the high oxygen content of thyroid tissue is a deterrent to the growth of malignant cells owing to their relatively anaerobic metabolism (Warburg⁹). The fact that exogenous neoplasms are more common in the adenomatous gland suggests that the structurally altered gland is more vulnerable to circulating tumor cells than the normal gland. It may be that the changed vascular condition favors the arrest of emboli, or that the abnormal thyroid tissue with its lower oxygen content offers a metabolically more suitable environment to the tumor cells. This phase of the subject has been thoughtfully considered and elucidated by Willis.¹ He has suggested also that other factors play a rôle. Since less common tumors such as melanoblastoma and lung tumors are relatively more common in the thyroid gland, it may be that their cells possess some special affinity for the thyroid gland.

Marked metabolic changes as a result of metastatic lesions in the thyroid gland have been described, ranging from myxedema (Willis¹) to toxic or exophthalmic goiter (Mori,¹⁰ H'Doubler,⁶ Welti and Huguenin,⁷ and Weiskittel¹¹). In only 2 of our cases were there any symptoms suggestive of hyperthyroidism and in only 1 was there an elevation of the basal metabolic rate.

Metastatic lesions in the thyroid gland, although comparatively rare, probably occur more frequently than is generally suspected. Sudden changes in the size of the thyroid gland of a person known to be suffering from malignant disease elsewhere in the body, are suggestive of metastatic lesions in the thyroid gland. On rare occasions the removal of a goitrous gland may reveal the correct diagnosis in a patient suffering from a wholly unsuspected malignant process.

4. Dehydrate with graded alcohols, 70 per cent, 80 per cent, 95 per cent, in the usual manner.
5. Complete dehydration of blocks with normal butyl alcohol, two changes of 4 hours each. (Any other routine method, such as absolute alcohol, xylol, acetone or dioxane [diethylene dioxide], is equally satisfactory.)
6. Embed in paraffin.
7. Cut sections, fix on slides, deparaffinize and hydrate to distilled water.
8. Place sections in *chromium chloride mordant* for 2 hours or longer. (Twenty-four and 48 hours in the mordant have caused no change in staining qualities.)
9. Rinse in distilled water.
10. Place sections in 0.25 per cent aqueous potassium permanganate solution for 10 to 15 minutes.
11. Rinse in distilled water.
12. Bleach in 5 per cent oxalic acid (until sections have lost brown tinge).
13. Rinse in distilled water.
14. Stain with Mallory's phosphotungstic acid hematoxylin 6 to 12 hours. (If Mallory's newer formula is used, potassium permanganate oxidation is necessary, as in his original formula.)
15. Decolorize in 95 per cent alcohol, two to three changes.
16. Dehydrate, clear and mount.

The *chromium chloride mordant* is prepared by dissolving 5 gm. of chromium chloride, green crystals ($\text{CrCl}_3 \cdot \text{XH}_2\text{O}$),* in 100 cc. of distilled water and adding 5 cc. of glacial acetic acid. This makes a dark green solution, which on standing takes on a dark purple-black color. This change is of no significance and the solution is usable after many weeks of standing. The cost is considerably less than one tenth the cost of fluorochrome.

The results from use of this mordant on formaldehyde-fixed material in our hands have been equally as brilliant as the results of phosphotungstic acid hematoxylin stains used on material fixed in Zenker's fluid. The differential staining of glial fibrils, fibrin, connective tissue, nuclei and cells is completely retained. Of

* Obtainable from the General Chemical Company, New York, N.Y.

A MORDANT PREPARING FORMALDEHYDE-FIXED NEURAXIS TISSUE FOR PHOSPHOTUNGSTIC ACID HEMATOXYLIN STAINING *

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One of the most widely employed and consistently useful stains for the routine study of the neuraxis long has been Mallory's¹ phosphotungstic acid hematoxylin. This stain gives brilliant differential staining, familiar to all neuropathologists, on brain tissue fixed in Zenker's fluid. However, in most laboratories doing routine studies on human brains and cords it is inconvenient, if not technically undesirable, to use a number of fixatives. In this laboratory, fixation in a solution of formaldehyde before sectioning the brain has been used for some time, and mordants of various types have been used before staining.

Refixing the desired blocks of tissue in Zenker's solution has proven inadequate. For several years we have had quite satisfactory results from the phosphotungstic acid hematoxylin stain after formaldehyde fixation by following Kernohan's² suggestion and mordanting in Weigert's mordants I and II. This method consumes several added days in the preparation of the sections. In recent months the great increase in cost of fluorochrome ($\text{CrF}_3 \cdot 4\text{H}_2\text{O}$) and the difficulty in obtaining it has made it desirable to find some other method. One of us (J.P.M.) has conducted a number of experiments with various chromium salts as mordants and the following method has been found eminently satisfactory.

PROCEDURE

1. Fixation in 4 per cent aqueous solution of formaldehyde. (Tissue stored in this fixing fluid for several years has been found to give good results.)
2. Cut blocks not over 5 mm. in thickness.
3. Wash blocks in running water 6 to 12 hours.

* Received for publication August 16, 1940.

course, numerous other stains may be used on sections from the same block since the mordant is applied to the sections and not the block.

SUMMARY

A method is outlined for mordanting sections of neuraxis tissue, fixed in solutions of formaldehyde, to make them receptive to Mallory's phosphotungstic acid hematoxylin stain. The method is simple and lends itself to routine use. The mordant material—chromium chloride—is much more cheaply and easily obtained than fluorochrome.

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form the basis for the clinicopathologic picture somewhat inaccurately designated as "renal rickets."⁸ There have been reported also a few cases of chronic renal insufficiency in adults in which the parathyroid hyperplasia was as pronounced as that associated with "renal rickets" and in which there were also very extensive osseous changes.⁹⁻¹² Altogether, the skeletal changes occurring in these adult cases may be regarded as the adult counterpart of those occurring in "renal rickets," and, in both, a condition of secondary hyperparathyroidism (due to the secondary parathyroid hyperplasia) is evidently partially responsible for the evolution of the osseous changes.

The object of this paper, however, is to stress the fact that osseous lesions of a much milder type than those of the aforementioned group can almost regularly be found in adult cases of prolonged renal insufficiency. Furthermore, we shall show that these bone changes may be associated with only mild or moderate parathyroid hyperplasia and even, rarely, with no gross or microscopic parathyroid hyperplasia, so that it seems plausible to explain their development on another basis than secondary hyperparathyroidism. Such findings had been previously described only by Rutishauser and co-workers¹³⁻¹⁶ who, in a series of papers published between 1935 and 1938, have called attention to the regular occurrence of osseous changes in adults with chronic renal insufficiency as well as in animals with experimentally produced renal insufficiency. They too question the rôle of the parathyroids in the genesis of these mild changes and stress the importance of the acidosis which is constantly present.

OBSERVATIONS

The present study is based upon the clinical and postmortem data on 12 cases of chronic renal insufficiency in adults. The ages of the subjects ranged from 39 to 76 years. The underlying renal damage consisted either of chronic glomerulonephritis, or chronic ascending pyelonephritis, pyonephrosis or hydronephrosis, often secondary to hypertrophy of the prostate. In all instances the state of renal insufficiency had evidently existed for some time. Furthermore, in all of them, chemical examination of the blood revealed nonprotein nitrogen retention, and hyperphospha-

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OSSEOUS FINDINGS IN CHRONIC RENAL INSUFFICIENCY IN ADULTS *

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Now that the skeletal and renal changes which follow in the wake of primary, or idiopathic, parathyroid hyperfunctioning¹ are well understood, increasing attention is being paid to the fact that chronic renal insufficiency may itself be the point of departure for the development of profound pathologic changes in the parathyroid glands and skeletal system. In primary hyperparathyroidism, the parathyroid abnormality usually consists of an adenoma (limited as a rule to a single gland) but sometimes of hyperplasia involving all (theoretically four) parathyroids.² The parathyroid abnormality secondary to the metabolic alterations induced by chronic renal insufficiency also involves the theoretical four parathyroids.³⁻⁶ However, the histological appearance of the hyperplastic glands in this group is different from that in primary hyperparathyroidism upon the basis of parathyroid hyperplasia.⁷

In some instances of chronic renal insufficiency the secondary parathyroid hyperplasia reaches such proportions as to leave no doubt that the total clinical picture has been complicated by the hyperfunctioning of these glands. This hyperfunction, in turn, may instigate further damage to the kidneys and may also exert its influence on the osseous tissues, as in primary hyperparathyroidism. Indeed, the skeletal changes may even come to occupy the foreground of the clinical picture. Thus, in childhood, protracted renal insufficiency plus "secondary" hyperparathyroidism

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spongiosa was close-meshed and the trabeculae were thickened and distorted, and altogether the condition amounted to an osteosclerosis (Figs. 3 and 4). Microscopically, it could be observed that this osteosclerosis had developed through gradual accretion of new bone, reparative processes evidently having predominated over resorptive processes in the alternation between the two, which must have been going on for a long time. Indeed, what amounted to a "renal" osteosclerosis was present in 3 of our cases and was very pronounced in 1 of them, in which the renal disease was of 12 years' standing.

Parathyroids

In all but 1 case there was either moderate gross hypertrophy of the parathyroid glands with microscopic evidence of hyperplasia, or only microscopic hyperplasia without obvious enlargement of the glands. In the exceptional case, the parathyroids showed no evidence at all of hyperplasia. Nevertheless, this case, too, showed osseous changes sufficiently pronounced to justify placing it in the moderately advanced group. However, in no instance was there parathyroid enlargement of the degree present in the group mentioned⁸⁻¹²—the cases commonly classified under the head of "renal rickets" or its adult counterpart. The average weight of each parathyroid in those of our cases in which this weight was taken was 92 mg. per gland as compared with the normal average of approximately 30 mg. The most pronounced enlargement in this series was observed in a case of chronic glomerulonephritis of several years' duration with a history of an attack of acute nephritis 20 years previously. In this instance, the total weight of three parathyroid glands found was 467 mg., or, on the average, 156 mg. per gland. In accordance with the findings of Castleman and Mallory⁷ in cases of secondary parathyroid hyperplasia, the enlargement of the parathyroid glands was due almost entirely to hyperplasia of the chief cells, the individual cells remaining of approximately normal size.

DISCUSSION

The observations presented here on the development of secondary parathyroid hyperplasia in cases of chronic renal insufficiency

temia, hypocalcemia, and lowered CO₂-combining power of the serum.

The skeletal alterations in these cases could be classified as slight or only moderately advanced (detectable only microscopically), and far advanced (discernible even on gross examination of the bones).

Slight or Moderately Advanced Changes

As already implied, when the changes were of these degrees (as they usually were), the bones appeared normal grossly. The milder the osseous changes, the more likely they were to be restricted to spongy trabeculae, the trabeculae of vertebral bodies showing them if they were to be found at all. When slight (Fig. 1), the changes consisted of the presence of scattered Howship's or resorption lacunae on the surfaces of the spongy trabeculae and even on the walls of their vascular canals. These lacunae were usually found filled with connective tissue cells and often also presented some multinucleated osteoclasts. Here and there, an erosion space showed some evidence of repair in the form of osteoblasts lining its surface, but there was no significant evidence of bone formation.

When the changes were definitely more advanced (Fig. 2), the manifestations included not only the formation of Howship's lacunae here and there on the trabeculae but also the encirclement and extensive perforation of the trabeculae by tracts of connective tissue. In addition, the cortical bone showed changes, in the form of enlargement and erosion of haversian canals. Side by side with these resorptive changes there could already be noted, in these cases, contrasting changes of a reparative nature, in the form of new-bone apposition upon the spongy trabeculae. However, in these moderately advanced cases, the reparative process was as yet slight, so that the bones as a whole still tended toward porosis.

Far Advanced Changes

Occasionally, and specifically when the renal insufficiency had been very protracted and had fluctuated, the architecture of the spongy bone was found altered, even grossly. In these cases, the

microscopic or mild gross evidences of hyperplasia, we are not convinced that a secondary parathyroid hyperfunction necessarily underlies the osseous changes which we have described. Indeed, if it did, it would be difficult to correlate the tendency, emphasized by both Rutishauser and co-workers¹³⁻¹⁶ and ourselves²¹ to osteosclerosis, in these cases, with the known osteoporotic effect of parathyroid hyperfunction in human beings. It is true that one might oppose here certain experimental observations on the effects of protracted injection of parathormone in rats. One of us (H. L. J.²²) has observed, as have others, that under these conditions an osteosclerosis may develop in rats. However, it is doubtful whether a valid comparison can be drawn between these experimental observations of osteosclerosis in rats and the osteosclerosis in adults with chronic renal insufficiency.

Albright, Drake and Sulkowitch¹⁰ have even expressed doubt that the osseous changes in their case representing the adult counterpart of "renal rickets" were caused by hyperparathyroidism or hyperfunctioning of the hyperplastic parathyroids. They believed that metabolic study of their case indicated that excretion of phosphates in excess into the gastro-intestinal tract prevented absorption of calcium from the intestines and led to decalcification in the bones. Rutishauser has consistently emphasized the importance of acidosis in the production of these changes and has minimized the rôle of parathyroid hyperfunction in this connection. It is pertinent here to refer to the experiments of Jaffe, Bodansky, and Chandler,²³ who were able to produce a state of chronic acidosis in dogs placed on a diet containing ammonium chloride. When this treatment was associated with the additional factor of insufficient calcium intake in the diet, there resulted the development of osseous changes which exactly simulated the osseous changes of hyperparathyroidism. The parathyroids, in these animals, showed no hyperplastic changes.

For the present we are in full accord with Rutishauser's idea that chronic acidosis resulting directly from renal insufficiency plays the important rôle in the instigation and maintenance of the changes in question. We believe that because of the deficient ability of the inadequate kidney to form base, the organism seems forced to excrete fixed base in order to be able to eliminate the

are in accord with previous reports. This hyperplasia may be due to the effect exerted upon the parathyroids by the hyperphosphatemia which is constantly present in progressive renal insufficiency,¹⁷ although it can also plausibly be argued that hypocalcemia rather than hyperphosphatemia may be the instigating factor.¹⁸ The pertinent question here is that of the possible relation of the parathyroid hyperplasia to the relatively mild bone changes we have noted in our cases of protracted renal insufficiency. In our opinion, these osseous changes are not altogether or necessarily ascribable to parathyroid hyperfunctioning. It may be said at once that there is as yet no undisputed method for the clinical estimation of parathyroid hyperfunction. Highman and Hamilton¹⁹ in 1937 maintained that by the method of Hamilton and Schwartz they were able to demonstrate increased activity of the parathyroid glands in 20 of 23 cases of chronic renal disease. However, Gilligan, Volk and Gargill²⁰ were not able to confirm these results, finding the test to be negative 18 out of 19 times in 15 patients with marked chronic renal insufficiency, thus indicating, if the test is valid, an absence of hyperparathyroidism. In 4 of the cases with negative results, death occurred in uremia, 1 being a case of "renal rickets." All 4 showed distinct secondary hyperplasia of the parathyroids *post mortem*. On the other hand, interestingly enough, Gilligan, Volk and Gargill found the test to be positive in 3 of 8 cases of active Paget's disease of bone and in 7 of 18 cases of thyrotoxicosis.

To what degree, therefore, a parathyroid hyperfunction participates in the production of the osseous changes in cases of chronic renal insufficiency in which the parathyroids show but little gross enlargement or only microscopic hyperplasia remains a question. (One cannot, of course, argue that hyperplasia of an organ is *necessarily* an indication of increased function; it may represent merely a reactive response to a stimulus.) We have no doubt that in cases of "renal rickets" with pronounced parathyroid enlargement, and in those cases of chronic renal insufficiency in adults associated with advanced skeletal changes and pronounced enlargement of the parathyroids, parathyroid hyperfunctioning can play a secondary rôle in the evolution of the osseous changes. At any rate, in regard to those of our cases which showed only

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acid end-products of metabolism. Among the fixed bases on which the organism draws are the calcium and magnesium stored in the bones, and hence the skeleton tends to become demineralized. With this abstraction of minerals, accompanied, as it probably is, by the exaggerating factor of deficient calcium absorption and utilization, reactive resorption and fibrosis of the skeleton is invited in turn. With periods of fluctuation of the renal insufficiency and acidosis, new-bone deposition occurs which, if it predominates to a great extent over the process of resorption, occasionally results in the occurrence of an unusual osteosclerosis.

SUMMARY

In cases of chronic renal insufficiency in adults, there almost regularly occur skeletal changes consisting of more or less pronounced fibroporotic resorption of bone accompanied by a varying amount of new-bone formation. The latter may occasionally be so pronounced as to result in an actual osteosclerosis, observable grossly as well as microscopically. This appears to occur in those cases in which the state of renal insufficiency is long protracted and possibly fluctuates, so that phases of bone resorption and new-bone formation alternate but the latter predominates.

In these cases, there is also secondary hyperplasia of the parathyroid glands. While in exceptional instances this may be sufficiently accentuated to suggest that hyperfunctioning of these hyperplastic parathyroids has been a factor in the production of the osseous changes, in most cases the hyperplasia is only mild or moderate and there is no definite evidence to support the assumption of a parathyroid hyperfunction. We believe, therefore, that in most cases of chronic renal insufficiency in adults, the osseous changes commonly found are not due to "secondary" or "renal" hyperparathyroidism but are a consequence of the chronic acidosis induced by the renal insufficiency.

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DESCRIPTION OF PLATES

PLATE 57

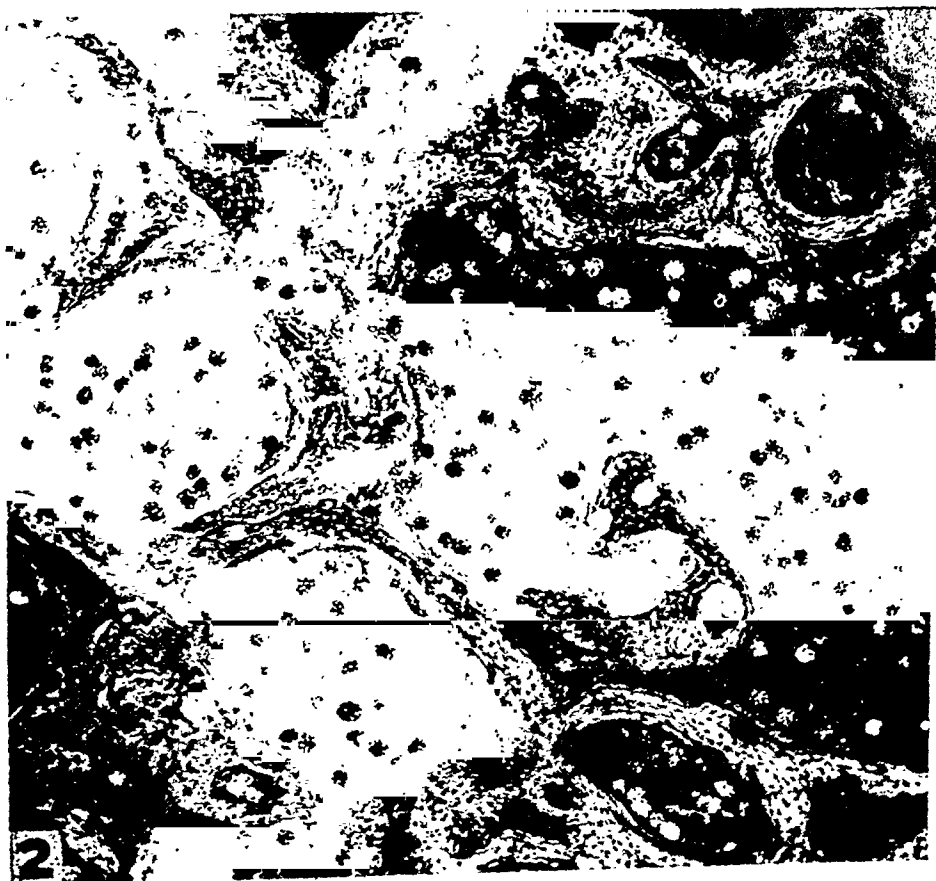
- FIG. 1. Spongiosa of a vertebral body from a case of chronic renal insufficiency in an adult, showing relatively mild changes in the form of tracts of connective tissue containing osteoclasts in the haversian canals and on the margins of trabeculae. $\times 50$.
- FIG. 2. Spongiosa of a vertebra, showing moderately pronounced changes in the direction of osteosclerosis and already including some distortion and thickening of the trabeculae, due to apposition of new bone, in addition to the evidences of resorption. $\times 50$.

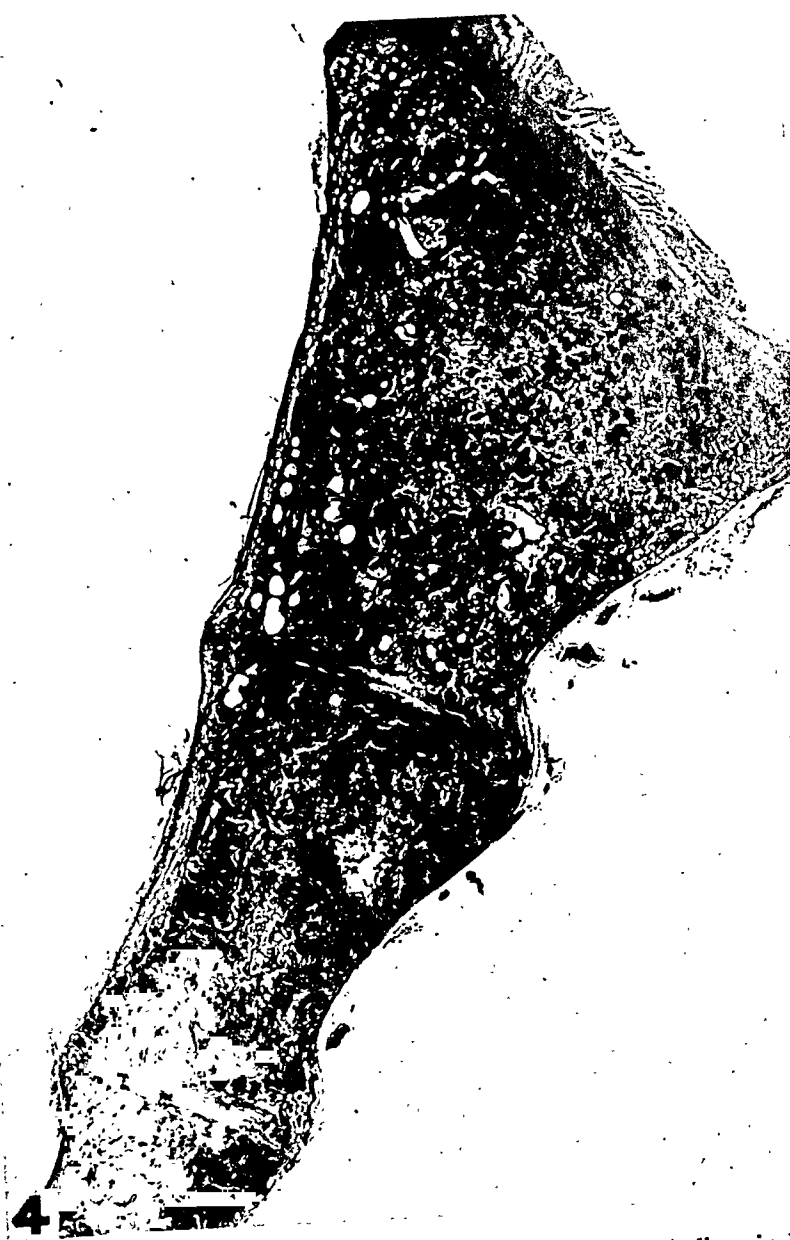
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PLATE 58

FIG. 3. Photograph of the three lowest lumbar vertebrae and the sacrum, in longitudinal section, to illustrate a "renal" osteosclerosis so pronounced as to be evident grossly.

FIG. 4. Low power photomicrograph of the sacrum illustrated in Figure 3 to show the closely compacted spongiosa. $\times 2\frac{1}{4}$.





METHOD

The procedure outlined by Gomori¹⁰ and by Takamatsu⁹ is based on the deposition of $\text{Ca}_3(\text{PO}_4)_2$ at the site of enzyme action, when a section of tissue is incubated with an organic phosphate ester in the presence of calcium ions. In order to insure optimum activity of the enzyme, constant conditions and maximum histological definition, the following procedure was used:

Tissues were fixed in 95 per cent alcohol and paraffin sections were mounted on slides. The paraffin was removed with xylol and the xylol with absolute alcohol. The sections were then dipped in a dilute collodion solution, allowed to dry, hardened in 90 per cent alcohol,¹⁰ and washed with distilled water. Alcohol under these conditions does not affect the stability of this enzyme. The sections were then transferred to substrate solution and incubated at 37° C. for 2 hours. Control serial sections were placed in a dilute (0.1 per cent) calcium nitrate solution. Stock solutions of 3.2 per cent sodium- β -glycerol-phosphate, 2 per cent calcium nitrate, 10 per cent sodium barbital, and 0.1 molar magnesium sulfate, were prepared. The solution used was made up by diluting 6 cc. of sodium- β -glycerol-phosphate, 9 cc. $\text{Ca}(\text{NO}_3)_2$, 6 cc. sodium barbital, and 6 cc. MgSO_4 to 60 cc., to give a final solution which was 0.01 molar with respect to glycerol phosphate and magnesium sulfate and had a P_H of 9. After incubation, each section was placed in the solution with its control and both stained for calcium by von Kossa's method, which replaces the calcium phosphate by metallic silver and gives a brown color at the site of phosphatase action. The sections were washed in absolute alcohol to remove the collodion, stained with hematoxylin and counterstained with light green. In the finished section the silver deposit (indicative of phosphatase) is brown, the cell nuclei blue and the cytoplasm green (Figs. 1-6).

Sodium barbital was used as a buffer since preliminary experiments showed very marked reduction and variability in the intensity of enzyme action in unbuffered solution. Magnesium, used in the concentration found to be optimal by O. Bodansky,¹² produced a slight increase in the intensity of the phosphatase reaction.

THE DISTRIBUTION OF PHOSPHATASE IN NORMAL TISSUES

The histochemical reaction showed the presence of large amounts of phosphatase in osteogenic tissues, renal epithelium and epithelium of the small intestine; that is, in tissues which form bone or are concerned with glucose absorption or elimination. In addition, small or moderate amounts of phosphatase were present in the endothelium of the blood vessels of most organs,

A HISTOCHEMICAL STUDY OF THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN VARIOUS NORMAL AND NEOPLASTIC TISSUES*

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Phosphatases having an *in vitro* P_H optimum of about 9 have been found in kidney, intestine, bone, and in smaller amounts in other tissues and in serum.¹ They have been stated to be involved in the absorption of glucose through the intestinal wall and reabsorption of glucose in the tubules of the kidney (Lundsgaard²⁻⁴), and in osteogenesis (Robison⁵ and Kay⁶). Quantitative analytical measurements for the estimation of serum phosphatase have been developed by A. Bodansky and Jaffe,⁷ and changes in serum phosphatase level were found in rickets, osteogenic sarcoma, obstructive jaundice, hyperthyroidism, and in other conditions.¹ Phosphatases were shown to differ by O. Bodansky,⁸ who observed that the activity of bone and kidney phosphatases was retarded by bile acids, whereas intestinal phosphatase was unaffected.

Recently the introduction of a histochemical method by Takamatsu⁹ and independently by Gomori,¹⁰ for demonstrating the presence of phosphatase in tissues, has made it possible to study the location of phosphatase in cells and its distribution in different tissues.

The present study was undertaken to determine the presence of phosphatase in various normal and malignant tissues, and especially its relation to bone formation in a transmissible osteogenic sarcoma of fowls.¹¹ The data on the distribution of phosphatase were accumulated before we were aware of the studies of Takamatsu.⁹ The histochemical method has also been used to study the inhibiting effect of various substances on the action of phosphatase in tissue sections.

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tissues were studied. The epithelium of the small intestine gave an intense phosphatase reaction (Fig. 6), most conspicuous in the upper layers of epithelial cells, while the deeper ones contained only a small amount of phosphatase. Autolyzed and desquamating epithelial cells also gave a marked reaction. A large amount of phosphatase was found in the endothelial cells of the submucosa. Occasional fibroblast-like cells of the submucosa likewise gave the reaction, while the muscle cells were free from phosphatase.

The epithelial cells of the large intestine did not give the reaction except a few cells in close proximity to the lumen. There were amorphous masses containing phosphatase in the lumen of the gut and also a few desquamated intestinal epithelial cells which contained only traces of phosphatase. Endothelial cells of the submucosa and of the lymph follicles almost invariably contained phosphatase and a thin film of phosphatase was found in the serosal layer.

Stomach. Epithelium of human gastric mucosa and that of the mouse embryo did not give the reaction.

Muscle. Adult human, and adult and embryonal mouse tissues were examined. Neither the heart nor the voluntary and smooth muscles examined contained phosphatase but the endothelium of capillaries gave a strong reaction.

Lung. Human adult, and mouse adult and embryonal tissues were examined. The usual alveolar epithelium did not contain phosphatase, but cuboidal alveolar epithelium contained some of this enzyme. In one specimen there was much edema fluid in the alveoli and this gave a faint phosphatase reaction. The endothelium, particularly of the large vessels, was well marked by this reaction. The epithelium of the bronchi and of the trachea contained small amounts of phosphatase. The cartilage of adult human trachea was phosphatase-free. Calcifying tracheal cartilage from an old individual contained no phosphatase about the calcium deposits. Much phosphatase was found in the basement membrane of the bronchial epithelium of an adult mouse. A small amount was found in the lumens of mucous glands and at the margin of this glandular epithelium in the trachea.

Liver. Adult human, chicken and mouse livers, and an embryonal mouse liver were examined. The endothelium of the sinu-

in certain nerve cells, in nerve fibers, in cells of the pituitary and in a few other types of cells.

Bone. The femur of a stillborn baby and bones of young chicks and of a mouse embryo were studied. Adult human bone could not be investigated because the usual procedure of decalcification results in the destruction of the phosphatase, but extracting the calcium salts from bones of a stillborn baby with diammonium citrate does not interfere with the demonstration of phosphatase.* The characteristic osteoblasts and the spindle-shaped periosteal fibroblast-like cells, wherever present, gave strong phosphatase reactions. Particularly instructive was the study of the embryonal bones. In the mesenchyme of the tail (Figs. 7 and 8) and leg rudiments large amounts of phosphatase were found in cells, which did not differ in appearance from the usual non-bone-forming mesenchymal cells. Proliferating cartilage cells in the chicken femur showed conspicuous phosphatase reactions. Cartilage in adult human trachea was free from phosphatase. Large amounts of phosphatase were found in the mandible and tooth anlage of a mouse embryo (Fig. 3).

Bone Marrow. Normal blood-forming elements, precursors of erythrocytes, granulocytes and megakaryocytes, did not give the phosphatase reaction. Some endothelial cells contained a small and variable amount of phosphatase. There were occasionally fine granules, indicating phosphatase action, scattered about the fat vacuoles.

Kidney. Adult human, and adult and embryonal mouse, tissues were examined. The cells of the convoluted tubules contained large amounts of phosphatase (Figs. 1 and 2). Their brush border was most intensely stained. The distal convoluted tubules, loops of Henle and collecting tubules contained no phosphatase, or traces only. The epithelium of Bowman's capsule gave an intense reaction if cuboidal, but was unstained if flat. The epithelium covering the glomerular tufts was free from phosphatase and only traces of phosphatase were found in the glomeruli.

Salivary Gland. In the lumen of a salivary gland about the acini, small amounts of phosphatase were demonstrated.

Intestine. Adult human, adult chicken and embryonal mouse

* Citrates have been used by J. Salk to dissolve calcium phosphate precipitates on which viruses have been adsorbed. (Personal communication.)

tained the largest amounts of phosphatase, which was present in smaller amounts in both the fascicular and glomerular layers. The relative intensity of staining reaction, however, varied in different fields. In another section, from a woman who died *post partum*, large amounts of phosphatase were found in cells of the zona reticularis, moderate amounts in cells of the zona fasciculata and small amounts in cells of the zona glomerulosa. Cells of the adrenal medulla were phosphatase-free.

Pituitary of the Human Adult. In a pituitary from a man 71 years of age, neurogenic cells of the posterior lobe, the capsule and capillaries contained much phosphatase, as did clumps of epithelial cells in the posterior lobe. In the anterior lobe, only the capillaries and occasional clumps of epithelial cells gave the reaction. The type of epithelial cells which gave the reaction has yet to be identified, but their presence in large numbers in the posterior lobe of the pituitary of an old man makes it probable that they are basophils.

Thyroid. Thyroid epithelium from a young adult did not contain phosphatase. The capillaries in the same section contained phosphatase.

Testis. In a section from a human adult, most spermatogenic cells contained a small amount and the basement membrane contained moderate amounts of phosphatase.

Prostate. In the prostate of a young adult 18 years of age, the epithelium was found to be free from phosphatase. Some muscle cells contained traces of phosphatase. In an adult 64 years of age, numerous epithelial cells of the prostatic acini contained slight or moderate amounts of phosphatase. The staining reaction of the cells lining the ducts was of the same intensity. There appeared to be less reaction in the hyperplastic than in the non-hyperplastic acini.

A phosphatase with an optimal P_H in the acid range, the so-called acid phosphatase, was present in large amounts in prostatic tissue. Attempts are being made to develop a staining technic for acid phosphatase characteristic of this organ.

Seminal Vesicles. In a section from a human adult the epithelium was found free from phosphatase; the endothelium contained much phosphatase and the muscle traces of it.

Uterus. The myometrium was free from phosphatase. The

soids and of other vessels gave a conspicuous phosphatase reaction. The entire capillary bed, as a rule, was mapped out by this reaction. The bile ducts and liver cells contained no phosphatase, or only traces, although occasionally fine granules were seen scattered throughout the section but not identified with any cell type. In human liver of a young adult the liver cells contained a large number of very fine granules.

In order to distinguish between endothelial and Kupffer's cells a chicken was given intravenous injections of fine carmine particles. The animal was killed, the liver and spleen were fixed in alcohol and tested for phosphatase by the technic described. In the sections thus prepared, Kupffer's cells were filled with carmine particles, but failed to give the phosphatase reaction; whereas the endothelial cells of the liver contained large amounts of phosphatase but were free from carmine (Fig. 4).

Spleen. Adult human, mouse and chicken spleens and the spleen of a mouse embryo were studied. The endothelium, as in other organs, was well marked by the phosphatase reaction but none of the other elements of the spleen gave the reaction. There was some phosphatase in fibroblast-like cells about the margins of the trabeculae. The spleens of the mice contained erythrogenic and myelogenic foci with megakaryocytes, but none of these cells was phosphatase-positive. In the chickens that received intravenous injections of carmine the histiocytes of the spleen were seen to contain large amounts of carmine and were free from phosphatase, whereas the endothelium gave a phosphatase reaction but was free from carmine.

Lymph Node. Endothelial cells of blood vessels contained much phosphatase, while endothelial cells of most lymph sinuses were free from phosphatase. Most lymphocytes were free from phosphatase, though occasional groups of lymphocytes contained small amounts. Also occasional endothelial cells of lymph sinuses contained traces of phosphatase. The occurrence of phosphatase in lymphocytes and endothelial cells of the lymph sinuses was variable, and, if present, the amount of enzyme was small.

Adrenal. In sections from a mouse embryo the capsule and some cells of the zona glomerulosa gave the phosphatase reaction; the remaining cells of the cortex and those of the medulla did not. In the adrenal of a human adult, cells of the zona reticularis con-

osteogenic character was not evident. The phosphatase tests showed, however, that they contained phosphatase in increasingly large quantities when cartilage or bone formation was taking place.

Fibrosarcoma. Cells of a transmissible fibrosarcoma (strain 11¹³), produced by a filterable agent, contained no phosphatase. This enzyme was also absent in cells of sarcoma and malignant endothelioma produced by another filterable agent.¹⁴ Transmissible sarcoma cells originating in a growth produced in the breast of a chicken by methylcholanthrene likewise failed to give the phosphatase reaction.

Fowl Leukosis. The leukemic cells in the liver, bone marrow and spleen of chickens injected with viruses of fowl leukosis (strains 1 and 2¹⁵) contained no phosphatase.

II. Mouse Tumors

Cells of a transmissible mouse sarcoma derived from mice that had been injected with methylcholanthrene failed to give the phosphatase reaction.

Adenocarcinomata of mice arising spontaneously in stock C₃H were likewise phosphatase-negative.

A spindle cell sarcoma, the osteogenic character of which was at first not recognized in the usual hematoxylin and eosin preparation, was found to contain very large amounts of phosphatase (Figs. 19 and 20). A careful study of the control sections showed, however, deposits of calcium granules in several places among the spindle-shaped, fibroblast-like osteogenic cells.

III. Rat Tumors

A liver carcinoma produced by feeding a rat with butter yellow (dimethyl-amino-azobenzene) was examined. The cytoplasm of the tumor cells contained a fine dust of brown granules. The necrotic parts of the tumor contained amorphous masses with granules. Most liver cells were free from phosphatase. The basement membranes of some cells, however, contained small amounts of phosphatase. The endothelial cells between the newly formed ductlike structures contained large amounts of phosphatase, while those of the sinuses of the relatively normal liver tissue contained none or only traces.

epithelial cells of the endometrium contained moderate amounts of phosphatase.

Bladder. The epithelium of the renal pelvis and of the urinary bladder of a mouse embryo gave a strong phosphatase reaction.

Pancreas. The epithelial cells of the pancreas from a mouse embryo and from a human adult contained no phosphatase.

Breast. In sections from the breast of a young woman *post partum*, and from another in the puerperium, the epithelial cells gave a strong phosphatase reaction, as did those in a fibroadenoma that will be described. There was also a small amount of phosphatase about the basement membrane and in the spindle-shaped, loose connective tissue cells between the glands. The colostrum contained only traces of phosphatase.

Ovary. In a section of ovary taken *post partum*, a very large amount of phosphatase was found in the corpus luteum, while the epithelium of adjacent primordial follicles contained none. Spindle-shaped, theca lutein cells surrounding the epithelial cells of the corpus luteum contained considerable amounts of phosphatase. The intensity of the phosphatase staining decreased gradually peripherally and none was found in the spindle-shaped cells distant from the corpus luteum. Capillaries almost invariably contained phosphatase; germinal epithelium was free from it. In a follicular cyst, granulosa cells lying free in the lumen contained no phosphatase, whereas several layers of polygonal and spindle-shaped cells about the cyst contained very large amounts of phosphatase. In a corpus albicans no phosphatase was found.

PHOSPHATASE IN TUMOR CELLS

I. Chicken Tumors

Osteogenic Sarcoma. The sarcoma studied is readily transmissible in chickens and is probably produced by a filterable agent.¹¹ Numerous samples were examined, some showing osteoblasts with almost no intercellular substance, and others showing mature bone, cartilage, and various stages of bone and cartilage formation by the malignant osteoblasts (Figs. 15-18). These osteoblasts were large round cells, slightly larger than large lymphocytes, with a large vesicular nucleus and a scant amount of cytoplasm. If seen with no osseous or cartilaginous matrix, their

the cells and it was not determined whether the phosphatase was free in the stroma or was in the fibroblast-like cells.

Perineural Fibroblastoma. The tumor cells of a malignant perineural fibroblastoma involving the sacral plexus showed no phosphatase.

Human Leukemia. Malignant lymphoid cells infiltrating the liver, spleen and other organs in a case of acute lymphoid leukemia contained no phosphatase.

DISCUSSION

The following observations indicate that the histochemical method of Takamatsu⁹ and Gomori¹⁰ for phosphatase is specific for this enzyme. When this method is followed, except for the presence of sodium- β -glycerol-phosphate, only tissues containing calcium are stained; no non-calcium-containing tissues give the von Kossa reaction. The histochemical reaction is most pronounced in those organs (kidney, intestine and bone) which have been shown by chemical determinations on aqueous extracts to contain large amounts of phosphatase.

By the use of this histochemical method, the presence of phosphatase in tissues and organs not previously known to contain the enzyme has been established, and its relation to cells clarified. Thus, in the kidney the phosphatase is contained in cells of the proximal convoluted tubules. The endothelium, the cells surrounding embryonal hair follicles (Figs. 9 and 10), the myelin sheath of nerves (Figs. 11 and 12), the spermatogenic cells of the testis, and cells of the adrenal cortex have been shown to contain phosphatase. It will now become of considerable interest to determine what rôle phosphatase plays in these tissues.

The occurrence of phosphatase in vascular endothelium, for example, is of considerable interest in relation to the origin of the serum phosphatase. Armstrong and Banting¹⁰ observed that removal of the viscera did not affect the serum phosphatase level and concluded that most of the serum phosphatase comes from bone. The presence of phosphatase in vascular endothelium may indicate either that this tissue contributes to the serum phosphatase, or that endothelial cells selectively remove phosphatase from the blood.

The presence of glycine had an inhibiting effect on the activity

IV. Human Tumors

Carcinoma of the Breast. Sections from 4 carcinomas of the human breast were examined. None contained phosphatase.

Fibro-adenoma. A section showing the characteristic appearance of a fibro-adenoma of the breast, contained brown granules in large amounts in the cells of both acini and ducts and particularly abundant about the basement membrane (Fig. 5).

Carcinoma of the Gastro-intestinal Tract. Sections from a carcinoma of the stomach and from 7 carcinomas of the large bowel were examined. The latter included a mucinous carcinoma and 3 well differentiated adenocarcinomas. Phosphatase was absent in the tumor cells but present in the endothelium and in occasional fibroblast-like cells of the stroma (Figs. 13 and 14).

In a tumor metastatic from the sigmoid colon to the liver, no phosphatase was found in the tumor cells, while the connective tissue and endothelial cells of the stroma contained large amounts.

Liposarcoma. The section was taken from a tumor on the thigh of a man. The tumor cells were free from phosphatase. The endothelial cells contained a moderate amount and scattered cells resembling histiocytes between the malignant cells contained a large amount of phosphatase.

Malignant Melanoma. Sections from two examples of metastatic melanoma were studied. One was in an axillary lymph node, with the primary site undetermined. The other, in the small intestine, was a metastasis from the vulva. Neither contained phosphatase.

Adamantinoma. In this tumor from the lower jaw of a man, the endothelial cells and many of the connective tissue stroma cells contained small amounts of phosphatase, while the tumor cells contained none.

Hypernephroma. The tumor cells of a hypernephroma of the kidney, surgically removed, were free from phosphatase, while the capillaries were well marked by brown granules indicative of this enzyme.

Wilms' Tumor. Most tumor cells contained moderate amounts of fine granules indicative of phosphatase, while the stroma contained moderate or large amounts. The quantity of phosphatase in the stroma was sufficient to produce enough granules to obscure

findings indicate that in the kidney the phosphatase is localized only in the proximal convoluted tubule,⁹⁻¹⁰ and if phlorhizin is also selectively localized in the proximal convoluted tubules its concentration might be high enough to inhibit the phosphorylation. Lundsgaard has himself observed a selective concentration of phlorhizin in the cortex of the kidney, and Ellinger and Lambrechts¹¹ demonstrated that phlorhizin dyes which produce glycosuria accumulate in the proximal convoluted tubules. The occurrence of large amounts of phosphatase in the lining epithelium of the small intestine also supports Lundsgaard's original hypothesis. None of the data presented by other investigators¹² directly contradicts Lundsgaard's original hypothesis.

Our results indicate that phosphatase occurs in cells of several different malignant tumors, the most important of which is osteogenic sarcoma. Malignant osteoblasts contain phosphatase even though there is no formation of cartilage or bone. This may be of some value in differentiating osteogenic from other tumors. For example, a sarcoma of the right arm, which was regarded as probable myosarcoma largely on the basis of its location, was found to contain large amounts of phosphatase. The malignant cells were fibroblast-like and because normal muscle cells are devoid of phosphatase it is unlikely that these malignant cells were derived from myoblasts.

Of four different strains of fowl tumors examined, only the cells of the osteogenic sarcoma contained phosphatase. Primitive blood cells from leukoses, like normal immature blood cells, were devoid of phosphatase.

Phosphatase was also found in a fibro-adenoma of the breast (Fig. 5), but not in four carcinomata of the breast. It is noteworthy that lactating breast also contains large amounts of phosphatase. These observations suggest that malignant transformation of mammary gland epithelium is accompanied by loss of this enzymatic function.

There are certain irregularities. Connective tissue, for example, is, as a rule, phosphatase-free. Occasionally, however, it contains small amounts of phosphatase. The fibrous connective tissue stroma of tumors often contained large amounts of phosphatase, even though the tumor cells contained none. These observations

of phosphatase. Thus, 0.25 molar glycine added to the substrate solution completely inhibited the action of phosphatase in bone, intestine and endothelium and almost completely inhibited phosphatase in the kidney in tissue sections. This is in agreement with the findings of O. Bodansky,¹² who demonstrated that glycine in concentrations higher than 0.00625 molar inhibited the action of kidney, intestine and bone phosphatase. This observation furnishes additional evidence for the specificity of the histochemical method.

No histochemical studies have hitherto been made on bone phosphatase, owing to the difficulty of decalcifying bone under conditions which did not destroy the enzyme. By the use of a 10 per cent solution of diammonium citrate, it was possible to decalcify bone without affecting the phosphatase. Kidney phosphatase was shown to be unaffected if the fixed tissues were kept for a week in diammonium citrate solution. Bone decalcified in this manner, however, gave poorer preparations than are usually obtained by other methods of decalcification. Additional data on bone phosphatase were obtained from developing mouse embryos, which can be cut with or without decalcification.

The method enables a differentiation between endothelial cells, on the one hand, and Kupffer's cells, histiocytes or monocytes, on the other. In one experiment Kupffer's cells and histiocytes of the spleen of a chick were marked by intravenous injection of carmine particles. Sections of liver and spleen showed no phosphatase in the carmine-laden cells, but the endothelial cells gave a good reaction (Fig. 4).

Lundsgaard²⁻⁴ observed that phlorhizin, in addition to producing glycosuria, inhibits the phosphorylation of glucose, as well as the absorption of glucose, from an intestinal loop. These observations led him to postulate that glucose absorption in the intestine and its reabsorption in the kidney tubule are associated with intermediate phosphorylation, and that the phosphatase subsequently dephosphorylates the glucose phosphate. Lundsgaard subsequently became more cautious about this hypothesis with respect to reabsorption of glucose in the kidney because the dose of phlorhizin which completely inhibited glucose reabsorption per gram of kidney tissue was less (about one-fifth) than that necessary to prevent esterification in tissue. The histochemical

sedimentable at high speed (approximately 27,000 r. p. m. for 1 hour), but upon autolysis the enzyme is liberated in an active, nonsedimentable form.

SUMMARY

The technic described by Takamatsu and by Gomori for the histochemical demonstration of phosphatase is specific for this enzyme.

Alkaline-phosphatase activity is characteristic of certain cells. Among normal cells, the epithelium of the small intestine and of proximal convoluted tubules, osteoblasts, and endothelium are particularly rich in phosphatase.

Of the tumors studied, phosphatase is present in conspicuous amounts in the malignant osteoblasts of a transmissible chicken sarcoma, and in an osteogenic tumor of the mouse. In the osteogenic chicken sarcoma, phosphatase is particularly abundant about the sites of bony and cartilaginous deposits. In three non-bone-forming strains of transmissible chicken sarcoma, phosphatase is absent.

Human fibro-adenoma of the breast contains much phosphatase, as does lactating breast, while this enzyme is absent in carcinoma of the breast.

In the liver and spleen, endothelial cells alone contain phosphatase. Kupffer's cells and histiocytes do not contain phosphatase.

The presence of phosphatase in normal and malignant osteoblasts supports the view that this enzyme is important in bone formation and may aid in the histological identification of osteogenic cells.

The presence of large amounts of phosphatase in the intestinal epithelium is in accord with the views of Lundsgaard on the relation of glucose absorption to phosphorylation. Similarly, its presence in the proximal convoluted tubules of the kidney reopens the question of a similar mechanism explaining the reabsorption of the glucose secreted by the glomeruli.

Glycine, a known inhibitor of phosphatase action, inhibits also the phosphatase reaction in tissue sections.

NOTE: The authors thank Oscar Bodansky for many suggestions, and Fred Stewart for much of the pathological material used in these studies.

suggest that young or proliferating cells contain more phosphatase than adult or resting cells. The phosphatase content of cells of the adrenal cortex varied greatly in different individuals and it is possible that this was due to changes in the functional state of the gland.

Muscle cells, as a rule, are phosphatase-free. Occasionally the sarcolemma takes the phosphatase stain, so that in cross section muscle fibers are surrounded by a bright brown honeycomb marking the sarcolemma sheaths.

Our observations with normal tissues are in essential agreement with those of Takamatsu⁹ and of Gomori,¹⁰ but there are minor discrepancies which will have to be cleared up by future work. For example, Takamatsu found that fibroblasts were free from phosphatase whereas in some of our sections these cells gave a definite phosphatase reaction. Takamatsu described strong phosphatase reactions in the epithelium of the large intestine, which were not seen in our preparations.

It is significant, as Takamatsu⁹ noted, that endothelium of lymph vessels is phosphatase-free, while that of vascular endothelium contains phosphatase. This may be interpreted by assuming that the phosphatase of endothelium of blood vessels is derived from the plasma phosphatase which is absorbed by or deposited on the cells, or that the reaction is subtle enough to distinguish between endothelium of blood and of lymph vessels.

The observation that cells of a liver tumor induced in a rat by the feeding of butter yellow contained phosphatase was unexpected because liver and bile duct epithelium do not contain the enzyme.

It is noteworthy that embryonal precursors of phosphatase-containing adult cells also contain large amounts of phosphatase at the stage of embryonal development when there is no evidence of functional activity. Robison⁵ has shown that mesenchyme of the mandibular anlage, incubated *in vitro* in tissue cultures, assumes histiogenic activity.

The histochemical reaction indicates that phosphatase is localized in the cytoplasm of cells. There is nothing known about its relation to other substances present in the cell. Recent observations¹⁰ indicate that the enzyme is bound to a substance

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DESCRIPTION OF PLATES

All sections were counterstained with hematoxylin and light green. The granules demonstrating the presence of phosphate appear brown or black in the color photomicrographs.

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PLATE 59

- FIG. 1. Mouse kidney. $\times 600$.
- FIG. 2. Mouse kidney, control. $\times 600$.
- FIG. 3. Mandible and tooth anlage of a mouse embryo measuring 20 mm. in length. $\times 98$.
- FIG. 4. Chicken liver (carmine particles injected ante mortem). $\times 300$.
- FIG. 5. Fibro-adenoma of the breast (human). $\times 98$.
- FIG. 6. Small intestine of a mouse embryo. $\times 67$.

J. S. Hooley, G. Hathaway, Jr., T. C. Gerwig, Jr., and C. M. Landmesser assisted in this study. The color photomicrographs were taken by Charles Breedis.

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PLATE 62

- FIG. 7. Oxygenic mesenchyme of the snail-ve of the tail of a mouse embryo measuring 20 mm. in length. $\times 130$.
- FIG. 8. Control section for that shown in Figure 7. $\times 130$.
- FIG. 9. Hair follicle of the scalp of a mouse embryo. $\times 180$.
- FIG. 10. Control section for that shown in Figure 9. $\times 180$.
- FIG. 11. Myelinated nerve fiber from chicken. $\times 330$.
- FIG. 12. Control section for that shown in Figure 11. $\times 330$.
- FIG. 13. Carcinoma of rectum (human). $\times 300$.
- FIG. 14. Control section for that shown in Figure 13. $\times 300$.

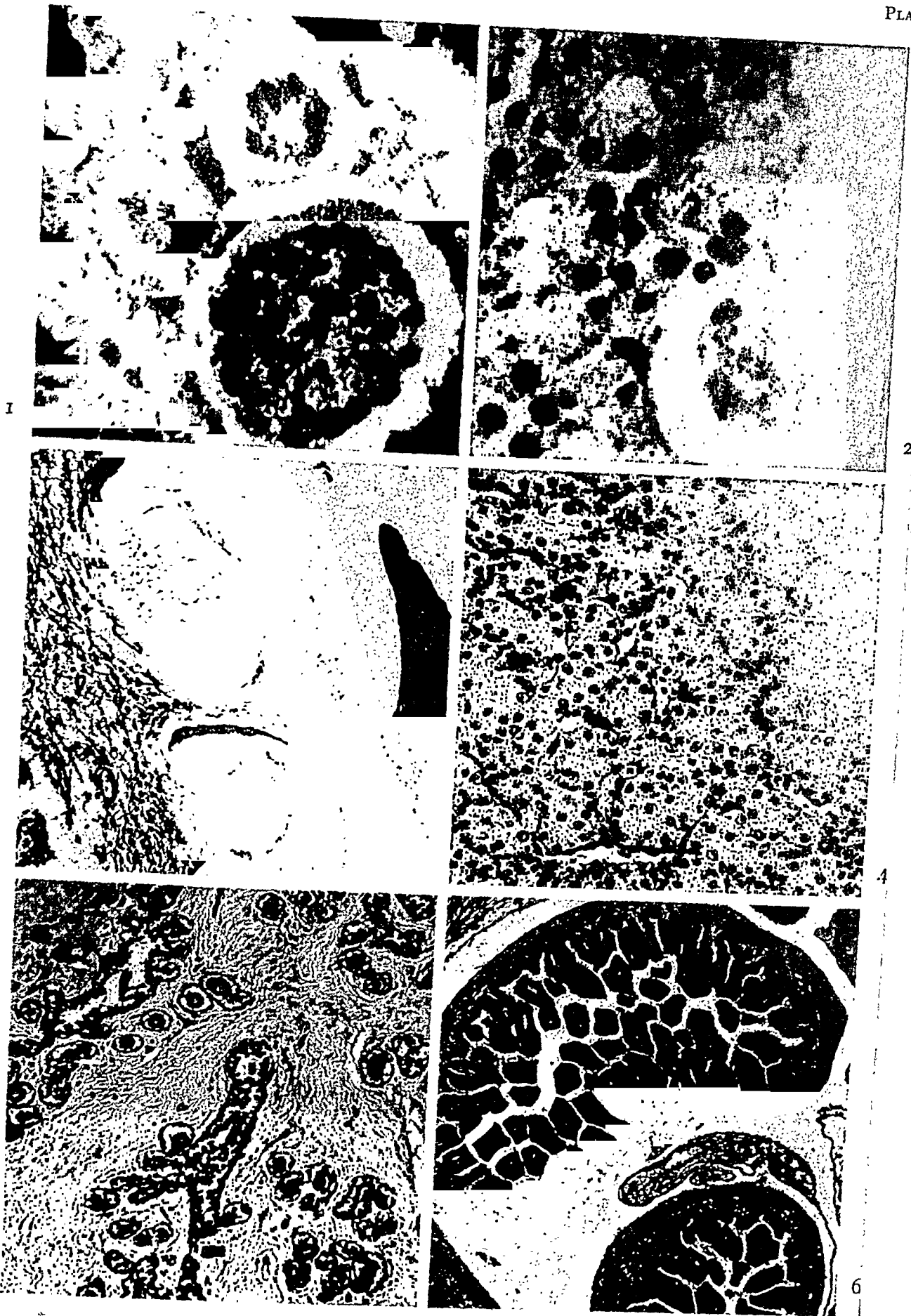


PLATE 61

FIG. 15. Osteogenic chicken sarcoma. $\times 100$.

FIG. 16. Control section for that shown in Figure 15. $\times 100$.

FIG. 17. Osteogenic chicken sarcoma. $\times 100$.

FIG. 18. Control section for that shown in Figure 17. $\times 100$.

FIG. 19. Osteogenic fibrosarcoma in a mouse. $\times 150$.

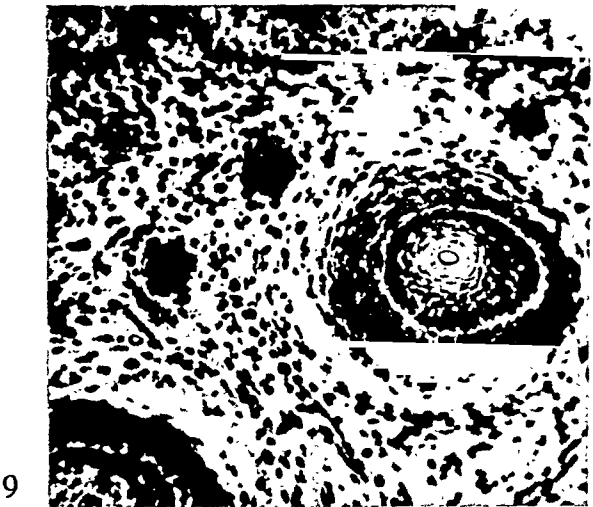
FIG. 20. Control section for that shown in Figure 19. $\times 150$.



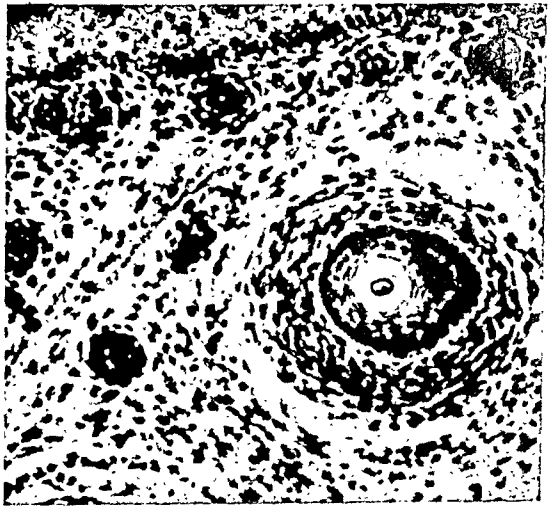
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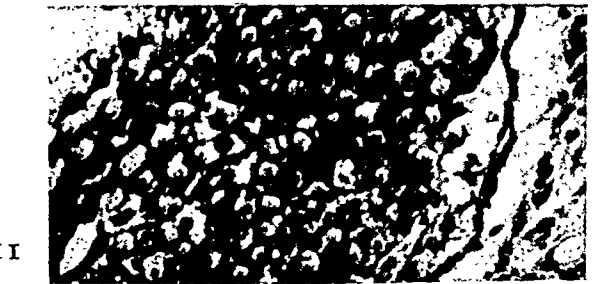
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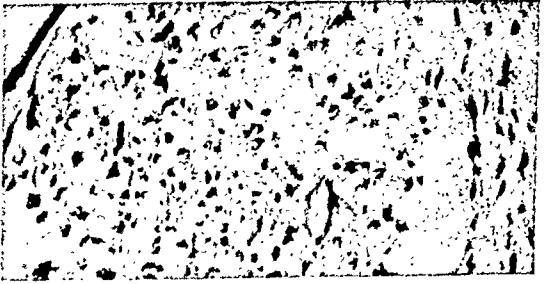
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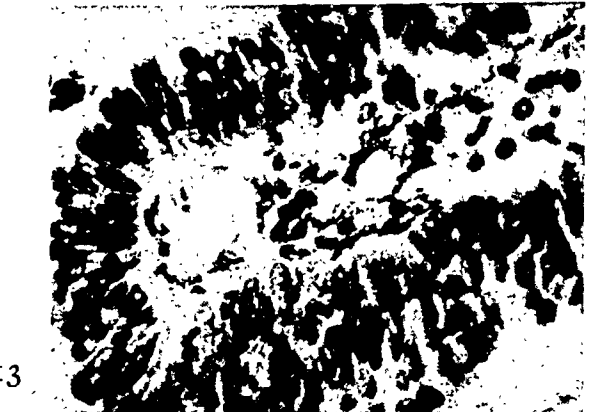
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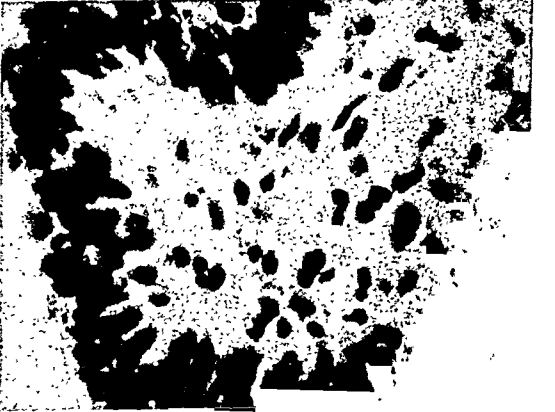
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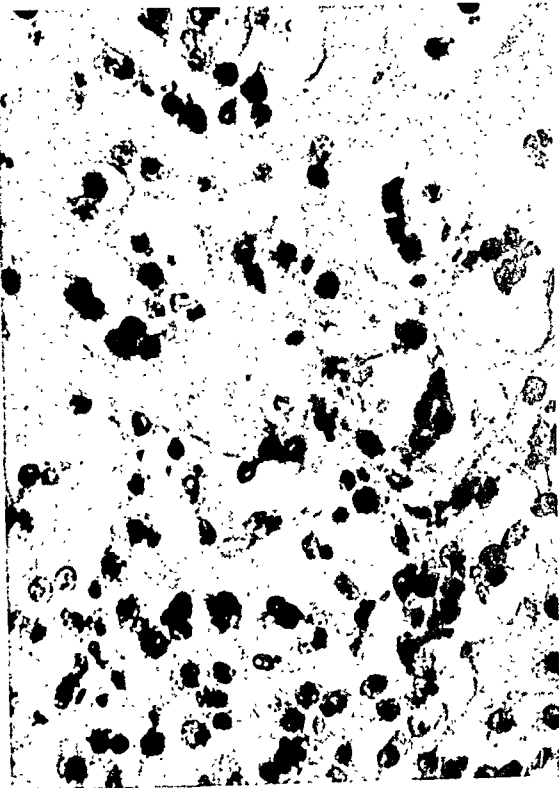


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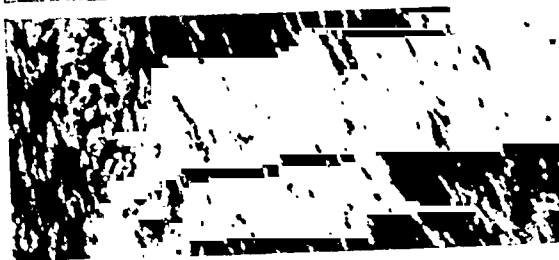
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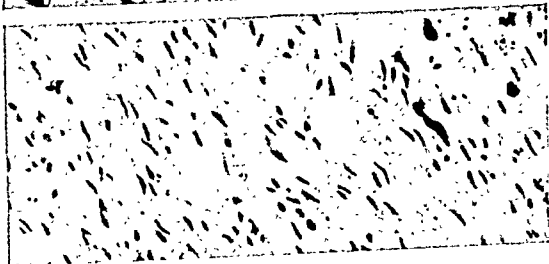
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they were 50 to 54 days old. All but 2 survived without evidence of intercurrent disease until such time as the needs of the experiment required their sacrifice.

The rats were divided into two major groups; group I received treatment with 200 kv. and group II with 1000 kv. roentgen rays. Each of these larger groups was further subdivided into six small subgroups, one of which (subgroup A) had no treatment and was retained for control purposes. Rats of the remaining subgroups were immobilized on a Y-shaped plywood board so that their hind extremities were abducted from each other and each rat was given a single dose of 600 r (subgroup B), 1200 r (subgroup C), 1800 r (subgroup D), 2400 r (subgroup E), or 3000 r (subgroup F), directed at the lateral aspect of the right thigh. In this manner 6 to 10 animals in each subgroup received equal doses from either machine. All dosages were measured in air with a Victoreen ionization chamber which was frequently checked with two others. The rats were then sacrificed by decapitation at prearranged intervals from 1 week to 19 weeks after irradiation.

The characteristics of the two machines utilized were as follows:

GROUP I PICKER-WAITE MACHINE		GROUP II VAN DER GRAAF GENERATOR
200,000 volts	Constant potential	1,000,000 volts
20 cm.	Distance	50 cm.
20 milliamper.	Current	0.85 milliamper.
0.25 mm. copper, 1.0 mm. aluminum	Filtration	3.0 mm. lead, 8.0 mm. copper
0.75 mm.	Copper half value layer	10.5 mm.
300 r/min.	Intensity	133 r/min.
10 cm.	Cone	10 cm.

Directly following sacrifice a strip of skin, including subcutaneous tissue and superficial muscle from the treated side of the thigh, was removed. Unfortunately, in the original experiments no material was excised from the medial aspect of the limb so that the effect of the "exit dose" could not be observed. The skin was immediately placed in Zenker's fluid with 5 per cent acetic acid and subsequently embedded in paraffin. Sections from all blocks were stained with phloxine-methylene blue. Con-

COMPARATIVE EXPERIMENTAL STUDIES OF 200 KILOVOLT AND 1000 KILOVOLT ROENTGEN RAYS*

III. THE BIOLOGIC EFFECT ON THE SKIN OF THE ALBINO RAT

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In view of the growing interest evoked by million volt roentgen therapy, a series of experiments has been performed in an attempt to establish the histologic changes produced by this means of irradiation under controlled conditions; and to compare these changes with those caused by roentgen rays obtained from a 200 kv. apparatus. We have chosen the albino rat as an experimental subject without particular purpose save for the facility of handling these animals under laboratory conditions. It is, however, admittedly unreasonable to interpret results obtained in any lower animal in terms of expected effect upon man, and our conclusions are therefore offered with this point distinctly in mind. Certain experiments^{1,2} have implied a greater degree of therapeutic efficiency of supervoltage (1000 kv.) rays than of high voltage (200 kv.) irradiation but clinical impressions have been somewhat varied.³⁻⁶ Previous comparative studies performed by us have demonstrated that the histologic changes effected in the growing epiphysis⁷ and the bone marrow⁸ of the rat have been essentially similar with either form of irradiation. The present investigation is concerned with the lesions produced in the skin of the thigh in the same animal.

METHODS

Since the procedure has been described in considerable detail in preceding reports,^{7,8} only a brief outline will be given in this publication. Ninety-five albino rats of the same strain were obtained shortly after weaning and were sustained upon the Sherman "BMS" regimen.⁹ At the time of application of irradiation

* The expenses of this study were defrayed in part by a grant from the Biological Engineering Fund of the Massachusetts Institute of Technology.

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and exhibited little angulation with the plane of the hair shaft. No sweat glands were present on the lateral aspect of the thigh. Elastic tissue stains demonstrated a fine intermeshed network throughout the upper portion of the corium, the general direction of the coarser fibrils being parallel with the surface.

200 Kilovolt Roentgen Rays

A dose of 600 r produced no histologic alteration in the skin of the rat. With 1200 r, however, a few dilated and congested capillaries appeared in the upper layer of the corium at about the third week. Similar changes appeared on occasion in the controls and therefore interpretation with any degree of assurance is not warranted. Nevertheless, despite the absence of any associated reaction, the congestion was considered to be the counterpart of clinical erythema.

During the first 2 weeks following doses of 1800 to 3000 r a more significant reaction appeared. There was slight swelling and vacuolization of the basal columnar layer and an accompanying increase in keratosis of the epidermis. Capillary congestion was apparent throughout the corium and there was an infiltration with lymphocytes, plasma cells and eosinophils arranged in a sprinkled fashion without characteristic distribution. Hair follicles exhibited slight swelling and vacuolization of sheath epithelium without remarkable variation in nuclear appearance. There was no evidence of epilation at this time. Subcutaneous blood vessels, fat, muscle and sebaceous glands were not appreciably affected.

Following the initial minimal reaction there was very rapid progression of events so that within the next 2 weeks maximal effects were apparent. There was complete epilation and, in the two subgroups receiving the highest dosage, complete epithelial denudation with ulceration and an intense inflammatory exudation in both corium and subcutaneous tissue (Fig. 2). At the edges of the eroded areas the surface epithelium was distinctly abnormal. Instead of two to four layers of epithelium there was marked thickening with as many as eight to twelve layers of cells. Deeper squamous cells exhibited vacuolization, hyperchromatism and, frequently, increased mitotic activity. In the upper three fourths of the epidermis there was an abundant

nective tissue and elastic tissue stains were applied when necessary for interpretation.

OBSERVATIONS

The material permitted histologic study of the effects of irradiation at graded intervals up to 5 months after the application of roentgen therapy. In addition it was possible to contrast the lesions produced by equal doses, as measured in roentgens, of rays developed by the high voltage and supervoltage machines.

Normal Rat Skin

The skin of the albino rat differs in several important respects from that of the human being. The epidermis in the region studied consisted usually of only two to four layers of squamous epithelium, over which a layer of partially desquamated keratinized material was loosely attached. Evidence of keratinization was present in the layer immediately overlying the basal columnar cells and the superficial layers were fully keratinized although they frequently contained stainable nuclei. Probably as the result of fixation, the surface was thrown into an irregular serrated outline (Fig. 1) but no recognizable epidermal papillae or rete pegs were present. The corium, except for a very narrow, finely fibrillar, subepidermal zone, consisted of irregularly distributed wavy bundles of collagen, among which a few thin-walled capillaries could be detected. The nethermost portion of the collagenous zone blended with a region composed predominantly of fatty tissue, in which were observed variable numbers of blood channels, still relatively thin-walled. This fatty zone was sharply demarcated on its deeper aspect by a thin layer of striated voluntary muscle. Penetrating the corium to a point variously proximate to the muscle were innumerable slender hair follicles arranged in a parallel manner. The bases or bulbs of the follicles were all situated in the fatty portion of the corium. Follicular walls exhibited stratification not unlike that observed in human hair although the thickness of the individual layers was considerably less. Each follicle at a point about midway through the corium gave rise to one or more oil gland appendages. The sebaceous glands consisted of cells with foamy cytoplasm indistinguishable from those in human skin but they were much smaller

of curlicue-like fragments. Their arrangement was usually perpendicular to or radiating from the surface plane instead of parallel, as is normally the case.

In general, following the fifth to sixth week after irradiation, the inflammatory reaction subsided and the denuded epithelium was regenerated. There were now, however, permanent epithelial alterations (Fig. 3). The epidermis was uniformly thickened, consisting of six to twelve layers of squamous cells, the superficial one half to one third of which were filled with dark-staining eleidin granules. Although somewhat hyperchromatic and pyknotic, stainable nuclei persisted within the most superficial cells. There was a moderately increased keratosis.¹⁰ Hair follicle regeneration was scant, most of the follicles retaining the nonstratified stumplike appearance previously described. Such follicular remnants were rudimentary and had the appearance of exaggerated epithelial papillae (Fig. 4). The few follicles which were reestablished were narrow and somewhat dwarfed in appearance. They contained normally arranged sheath layers and hair shafts, however, and small but well differentiated sebaceous glands were appended. Such restoration of hair as was grossly visible was sparse, fine and stunted.

In the corium the connective tissue exhibited a sharp division into two distinct layers. For a variable depth immediately subjacent to the epidermis there was diminished staining power and lessened collagenization, with increased prominence of fibrillae. In this region numerous thin-walled capillaries were evident, many of which had direct contiguity with the basement membrane of the surface epithelium. The latter was frequently separated from the underlying corium by narrow crevices, within which were observed innumerable tiny fibrillae forming fine sievelike fenestrations (Fig. 5). These maintained a degree of connection between the epidermis and cutis. Scattered throughout the superficial zone, frequently lying within the subepidermal crevices, were many large and elongated, stellate, basophilic fibroblasts.¹¹ Mitotic figures and multiple nuclei were occasionally noted in these elements.¹⁰

In the deeper stratum of the corium, collagen bundles were normally homogeneous but lacked the wavy, intertwining qualities apparent in untreated integument. The bundles were gen-

deposit of keratohyalin but even within the most superficial strata, nuclei were regularly visible. The inverted cuplike region of the hair follicle base disappeared and the lowermost portion of the persistent follicle became blunt and truncated. Sebaceous glands had disappeared at this stage and unfortunately none of our sections showed any intermediary phases of disintegration. Representing specialized follicular appendages, their existence was intimately related with the functional status of the follicle.

In this phase, marked congestion of blood vessels in all cutaneous layers was demonstrated, particularly in the subepidermal zone. Although there was a moderate degree of endothelial swelling with obstruction into the lumen, no proliferative activity was noted at this time. There was a perivascular accumulation of inflammatory elements similar to that noted elsewhere in the corium and occasionally thrombosis and mural necrosis. The corium and subcutaneous tissues were widely infiltrated by varied proportions of polymorphonuclears, eosinophils, monocytes and lymphoid elements, the latter usually preponderating. A similar but less well marked reaction was present in the deep panniculus and in this region fat cells were shrunken and finely vacuolated, and their nuclei had become vesicular. Often they formed clusters surrounded by vacuolated mononucleated phagocytes and but rarely by multinucleated foreign body giant cells. The lesion represented incomplete fat necrosis.

Collagen bundles in the corium were separated by marked edema and were intrinsically swollen, fibrillar and much less wavy than usual. This was particularly evident in the more superficial region. Immediately subjacent to uneroded epithelium, accumulation of excess tissue fluid caused separation from the underlying corium, forming small fluid-filled, bleblike spaces. In the same region a few fibroblasts, divested of fibrillar matrix, lay free in the tissue spaces. These elements were swollen, stellate and contained abundant basophilic cytoplasm. Their nuclei were large, irregular and vesicular. Deeper in the corium the connective tissue, although not obviously affected, exhibited a homogeneous, dull, blocklike reaction to the various stains utilized. There was greater prominence of elastic tissue fibrils, which were also frequently split. In regions of more intense reaction and hair follicle destruction, the elastic tissue appeared as small clusters

layed until about 1 to 2 weeks later. Higher doses, however, disclosed obvious variations in the effects caused by the two machines. The initial cutaneous response was essentially the same for both but again appeared 1 or 2 weeks later following supervoltage irradiation. This was in accordance with the clinical observations recorded by Stone.⁵ Subsequent to the initial skin reaction, however, those animals receiving 1800 r or more, except for partial epilation, exhibited no further progression but exhibited instead a fairly prompt regression to a state indistinguishable from the normal (Fig. 6). In view of the albinism of the subjects, pigmentary stigmata were naturally not apparent. It was quite obvious that large amounts of roentgens (1800 to 3000 r), derived from 200 kv. radiations, caused profound destructive cutaneous alterations which, with subsidence, left permanent residua. On the contrary, equivalent doses of 1000 kv. rays caused, at most, insignificant changes which were promptly and permanently rectified. Animals exposed to supervoltage rays were observed for a considerably longer period than those in the high voltage group, so that it was evident that a delay in response could not explain the divergence in cutaneous reactions.

DISCUSSION

The cutaneous reaction to roentgen rays is one of the most obvious biologic effects of these elements. For almost 4 decades it has been utilized to a greater or less degree as an index of radiation dosage. Since the scope of deep radiotherapy has been sharply limited by the intensity of skin injury, any means of circumventing this "barrier" has considerable clinical value. The experimental investigations of Failla^{1,11} and the clinical observations of Dresser and co-workers^{3,4} have suggested supervoltage rays as a means of avoiding much of this difficulty. Stone,^{5,6} however, by clinical experimentation has concluded that no greater advantage appertains to supervoltage therapy than may be obtained with diligently controlled high voltage treatment. Since experimental observations upon the comparative effects of equal doses of the two types of rays upon bone marrow⁸ and the epiphysis⁷ showed no significant variations in the degree of alteration produced, the controversial question of relative skin sensitivity becomes an important one. Skin sections from our subjects have therefore been subjected to particularly detailed

erally parallel to the surface plane in contradistinction to the irregular distribution evident in the normal state. A few swollen, atypical fibroblasts were present. Elastic tissue was seemingly markedly increased in amount and the fibrils were coarsened and irregular. In the region intermediary between the two zones of the corium there were numerous condensations of small, curled fragments but in the subepithelial regions scarcely any elastic tissue was found. In contrast with the normal fine meshwork of elastic fibrils arranged obliquely or parallel with the surface, the damaged skin manifested extreme irregularity and the direction of the fibrillar distribution was more commonly at an angle or perpendicular to the surface.

Blood vessels in the deeper cutis and subcutaneum were not remarkably increased in number or prominence. About many there was a considerable degree of perivascular fibrosis and a few retained a loose collar of lymphocytes and monocytes. Fibrils resembling elastic tissue (Gitterfasern) were unusually prominent in the media and even more so in the adventitia of some of the arteries.¹² Endothelial elements were not noticeably altered nor were vascular occlusive processes evident.

A marked grade of fibrosis was observed in both the subcutaneous tissue and the fatty panniculus. In the latter there was extensive lobulation as the result of the increased fibrous trabeculation. A peculiarity, probably an artefact but sufficiently constant to be worthy of note, appeared in these sections. In contradistinction to the irregular surface (Fig. 1) present in normal or minimally irradiated skin sections, the heavily irradiated specimens showed a relatively smooth surface which was only slightly, if at all, serrated (Fig. 3). This feature was undoubtedly the result of the effect of the fixative upon structures containing damaged connective tissue elements.^{11, 12}

1000 Kilovolt Roentgen Rays

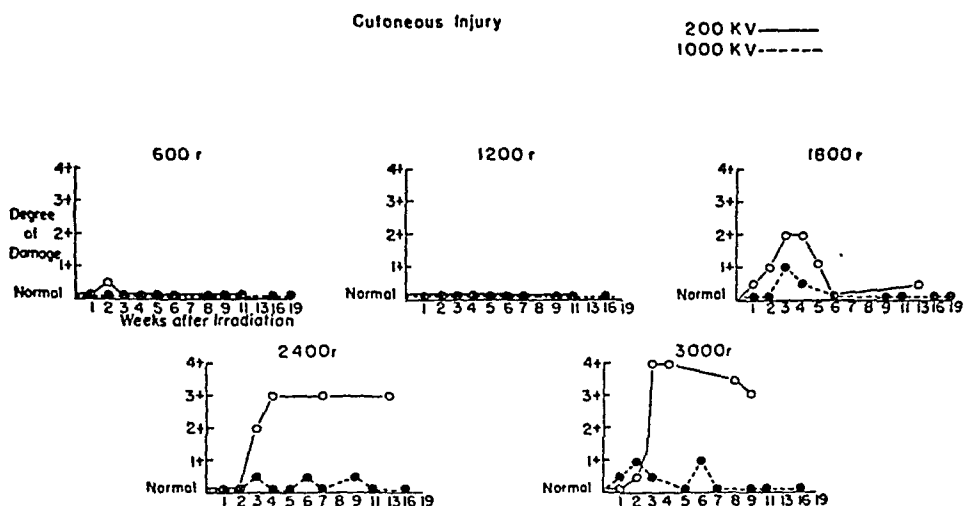
With dosages of irradiation up to 1200 r, the ultimate effects upon the skin did not differ significantly following either the high or supervoltage irradiation. Six hundred r from the 1000 kv. apparatus produced no recognizable changes whatever. With 1200 r superficial capillary congestion occurred, similar in all respects to that observed after the lower voltage rays, but de-

Some other cause for this variation must therefore be sought. The region treated, the age and metabolic status of the subjects, and the stock were all constant. Discrepancies could not be attributed to a difference in the biologic material utilized. Suspicion must then naturally fall upon the physical character of the roentgen rays applied. Failla¹ and Trump¹² have implied that the predominance of shorter wave lengths in the beam, developed by the supervoltage apparatus, produces maximum effects below the integument instead of at the point of surface impact, as occurs with the longer wave lengths produced at 200 kv. Our study has not included investigation of the tissues intermediate in depth between skin and bone and we are therefore unable to establish this point unequivocally. It is obvious, however, that the thickness of the irradiated parts was in all cases too small to make any diminution in the intensity of the primary beam an important factor.

We have not been able to confirm Stone's² clinical observations, from which he concluded that except for a delay in the appearance of erythema there was very little difference in the effect of the high and supervoltage radiation on the skin. Later observations⁶ demonstrated not only similarities in minor initial reactions, a feature with which our observations have been in accord, but in severe delayed reactions as well. It is reasonable to suppose that the dissimilarity between Stone's experiences and those which we have had may be attributed to the nature of the material utilized. The rat's skin is unquestionably a very different structure from that of the human being and it is possible that its reaction to irradiation might differ widely. Other influential factors might arise from variation in the thickness of the part treated and in the density of its component structures. Since "back-scatter" is of such significance in the development of tissue reactions and since this factor is closely related to the character and thickness of an irradiated part, it probably deserves major consideration in parallel comparisons of reactions in the rat and in the human being. Our material justifies interpretation only of the reactions observed in the rat. Comparative implications with the human being must be avoided.

In the rat, however, two mechanisms are possible. Either the

study. Each structural component has been individually investigated in all stages of the reaction and conclusions drawn from the composite whole. Text-Figure 1 offers graphic comparison of the degree of damage effected by either form of irradiation. In contrast to the similar results observed in the growing epiphysis and bone marrow regardless of the voltage, doses measured in roentgens equivalent to those derived from a 200 kv. source, which cause severe and permanent skin changes, will, when obtained from the supervoltage apparatus, cause only insignificant cutaneous damage.



Text-Figure 1. Graphic comparison of the degree of cutaneous injury produced by equal amounts of roentgens of the two voltages used.

It has been suggested that calibration of dosage with the Victoreen dosimeter permits the entrance of a physical error which may account for the discrepancy in results.¹³⁻¹⁶ According to this hypothesis a given dose of 1000 kv. rays measured in roentgens with the dosimeter would be equivalent to only approximately 75 per cent of an equal dose of 200 kv. rays determined by the same method. It is impossible to estimate the degree of biologic reaction obtained in this study in an accurate mathematical manner but it may be safely stated that the variation in the effect upon the skin was not 25 per cent, as anticipated by physical discrepancies, but as much as 200 to 300 per cent. It is believed, therefore, that the physical error inherent in dosimeter evaluation is inadequate to account for the degree of difference in reaction.

investigated in the supplementary series. In those animals unshielded by paraffin it was found that the lesion appearing on this portion of the extremity was much more severe than that noted on the lateral aspect. The gross and histologic appearance, in fact, was exactly similar to that evident on the lateral side of the sheathed limb. There was complete epilation, permanent cutaneous damage and often widespread ulceration. The inner thigh of the wax-sheathed rats likewise showed more damage than the outer, and in some instances the severity of the lesion exceeded even that observed with the highest experimental doses given from the 200 kv. generator.

The observations of increased cutaneous damage as the result of artificial displacement of integument to a subsurface position by means of a wax phantom and the greater severity of the injury inflicted upon the medial aspect of the unsheathed extremities are complementary. They lend considerable support to the belief that variations in skin changes effected by high and supervoltage roentgen rays cannot be attributable to a wave length effect.

SUMMARY AND CONCLUSIONS

Equal amounts of roentgen rays, as measured with the Victoreen dosimeter, were obtained from a 200 kv. and a 1000 kv. generator. Two large groups of albino rats were given graded amounts of irradiation to the skin of the thigh from either of these sources. The character of the histologic changes produced at periods of 1 to 19 weeks after irradiation are described.

It was noted that high dosages of 200 kv. rays (1800 to 3000 r) caused severe and permanent injury to the skin and its appendages. Equal doses of supervoltage rays, however, caused only mild transient morphologic changes. The differences apparent in the lesions produced by the two types of rays are far greater than the amount of error attributable to the Victoreen dosimeter. The hypothesis is therefore advanced that the discrepancy must then result from either a specific cutaneous tolerance for supervoltage rays (wave length effect) or an enhanced skin penetrability, inherent upon the physical qualities of the rays themselves, due fundamentally to the difference in the

difference in the physical quality of the supervoltage rays finds the rat's skin relatively insensitive (wave length effect) or the hypothesis of a greater subsurface ionization effect must be invoked. The second of these two can be established by elimination. The question of wave length effect has been subjected to further experiment.

Twelve additional rats were divided into two groups of six. To each of these was administered a dosage of 3000 r (1000 kv.) in a manner similar to that already described. In one group, however, at the suggestion of J. G. Trump, the treated extremities were closely ensheathed to a depth of 0.5 cm. with a coating consisting of equal parts of beeswax and paraffin. This substance had previously been shown to possess physical qualities relative to roentgen rays not unlike those exhibited by mammalian tissues. Thus 6 animals received 3000 r directly to uncovered skin and 6 others were given the same amount of irradiation through a 0.5 cm. wax medium.

The purpose of this procedure was to determine whether the skin of the rat possessed inherent resistance to supervoltage rays, or whether these rays provoked tissue reaction beneath the surface layer without causing injury at the point of initial impact. Since the wax phantom caused the skin to become a subsurface structure, in accordance with our earlier experiments,^{7,8} it might be expected to become as susceptible to 1000 kv. roentgen rays as the uncovered integument had been to 200 kv. rays.

Figures 7 and 8 exemplify the changes produced in both series at the peak of radiation reaction (4 weeks). It is quite obvious that the reaction produced in the unsheathed limb (Fig. 7) is relatively insignificant. Except for partial epilation on the lateral aspect of the thigh, no striking change is apparent. The paraffin-covered extremity (Fig. 8), on the other hand, exhibits not only complete epilation but in addition a profound cutaneous injury resulting in ulceration. Histologic modifications noted in this indirectly treated skin were quite similar to those already recorded following the administration of an equal number of roentgens from the 200 kv. machine.

The effect of the "exit dose" upon the skin of the medial aspect of the thigh, a feature neglected in the original experiments, was

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degrees of ionization produced by the two types of rays at the level of the skin.

By encasing the limb in a coating of beeswax and paraffin the skin was artificially transformed into a "subcutaneous" structure. It was then apparent that an amount of supervoltage rays which would cause only minimal damage to the skin under ordinary circumstances would, when applied through the medium of a layer of paraffin, produce a lesion similar to that found after like dosages of 200 kv. irradiation. It is believed, therefore, that the discrepancy in skin damage caused by the two types of rays cannot be the result of an inherent resistance of the epithelium to supervoltage rays.

It seems more reasonable to attribute this phenomenon to the fact that the ionization, to which the skin is subjected when irradiated by entering supervoltage rays, is different from the ionization which these rays produce some distance below the surface. Beneath the surface the ionization is probably more intense and contains relatively more low-energy secondary radiations. This is probably due to the fact that the "secondaries" produced by supervoltage rays are scattered predominantly in a forward direction so that a considerable thickness of tissue will be traversed before an equilibrium of primary and secondary ionization exists. In the case of the 200 kv. radiation this equilibrium is reached at a point much closer to the surface.

DESCRIPTION OF PLATES

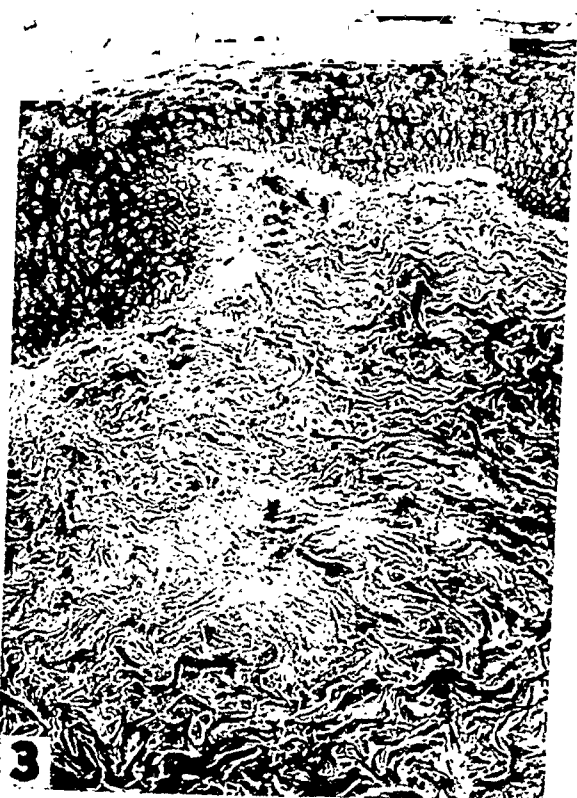
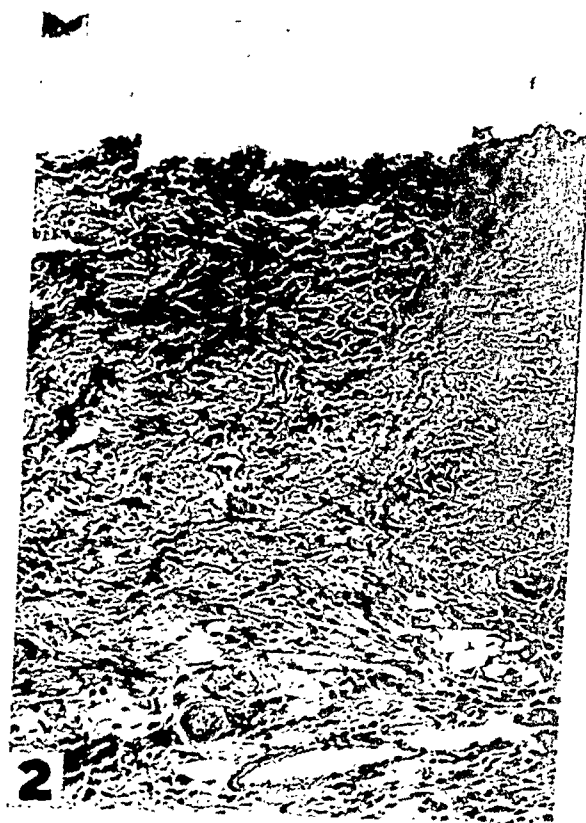
PLATE 6:

- FIG. 1. Normal rat skin. The thin epidermal layer is thrown into irregular serrations. There are numerous hair follicles, attached to each of which is a small sebaceous gland. $\times 60$.
- FIG. 2. Skin of a rat 19 days after irradiation with 5000 r (200 kv.). Surface epithelium is completely denuded and overlaid with a crust of partially necrotic exudate. Collagen is dull and blocklike in character and there is an intense infiltration with leukocytes. Hair follicles have been destroyed. $\times 60$.
- FIG. 3. Permanently damaged skin 47 days after treatment with 2200 r (200 kv.). There is marked thickening of the epidermis, which no longer exhibits the surface irregularity noted in the normal skin. Skin appendages are absent and there is dense blocklike thickening of the corium. $\times 60$.
- FIG. 4. Section from the edge of a heavily treated area (200 kv.) showing solidification of hair follicles with loss of normal stratification. Sebaceous glands have disappeared. The stumplike character of the residual follicles is evident. $\times 60$.

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PLATE 63

- FIG. 5. A high power view of the epidermal surface of the skin shown in Figure 3. The fenestrated, clublike expansion of superficial cornium from epidermis is apparent. Within the cornium are several large, bizarre fibroblasts. $\times 100$.
- FIG. 6. Skin section 21 days after the administration of 3000 r (1000 kv.). Except for a slight degree of keratosis the appearance is indistinguishable from that of untreated skin. $\times 60$.
- FIG. 7. The lateral aspect of the thigh of a rat 2 weeks after direct irradiation with 3000 r (1000 kv.). The cutaneous surface is intact and there is only partial epilation.
- FIG. 8. The lateral aspect of a thigh 2 weeks after the administration of 3000 r (1000 kv.) through the medium of a 0.5 cm. layer of beeswax and paraffin. There is complete epilation, the skin is opaque and dull in appearance, and there is a large area of ulceration.





quite possible that the displacement in different normal cells may also be different. A false impression might be obtained if a tumor cell of a type highly resistant to displacement by centrifugal force were compared with a normal cell of characteristically low resistance.

2. Emphasis was placed by Guyer and Claus on differences between the displaceability of the contents of carcinomatous cells implanted into the adrenal and that of cortical adrenal cells, both of which were centrifuged together. Their Figure 15 shows no perceptible displacement of contents of the carcinomatous cells, while their Figure 14 demonstrates marked displacement of the contents of the cortical cells. Adrenal cortical cells are particularly rich in lipids, probably more so than the carcinomatous cells. Guyer and Claus employed an air driven ultracentrifuge of the Beams's type. They made no mention of a temperature change, but if an increase in temperature did take place in their experiments during centrifugation it would lower the viscosity of lipid-rich cells more than that of cells containing less lipid.

3. In general, one would not expect malignant cells, capable of multiplication and invasion and youthful in some respects, to be stiffer throughout their substance than normal cells of the same source. One would look, on the contrary, for greater fluidity and lower viscosity. Employing the technic of microdissection, Chambers and Ludford (1932) were unable to discover any differences in consistency among the cells of two mammary gland carcinomata, one tar carcinoma and two sarcomata and normal cells in tissue cultures. But, unfortunately, this comparison also lacked in directness.

The purpose of our experiments was to secure further data on the comparative viscosities of the nuclear contents of malignant and normal cells of the same type.

MATERIAL

This is indicated in Table I. The study of mice has been supplemented by the examination of human tissues.* The tissues of three strains of mice were employed.

1. New Buffalo mice constituted the main series. All, except

* Most of the human material was obtained from the clinics in the Barnard Hospital, but we are grateful to other St. Louis hospitals for additional specimens.

ALTERATIONS IN NUCLEAR VISCOSITY DURING EXPERIMENTAL CARCINOGENESIS DETERMINED BY ULTRACENTRIFUGATION*

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Experiments by Guyer and Claus (1939) seem to indicate that the long sought fundamental difference between malignant and normal cells may be one of viscosity. They write: "Judging from the lack of displacement of cellular contents in tumor cells subjected to centrifugalization at extremely high speed, the protoplasm of cancer cells, notably that of carcinomas and adenofibromas, has a decidedly greater viscosity than that of normal tissue cells." They explain that "since the nuclear contents are as little displaced by the centrifuging as are the cytoplasmic, there would seem to be a general stiffening up of the entire cell substance. The only other alternative would be the highly improbable supposition that all of the inclusions of the tumor cells have become of the same specific gravity as the general cytoplasmic ground substance."

The lack of displacements and the displacements observed by Guyer and Claus are well described and illustrated. We do not question them; but, without further evidence, we are not ready to accept their conclusions for the following reasons:

1. In order to subject the malignant cells to the same centrifugal force as normal cells, they implanted carcinomatous cells into the adrenal, kidney, pancreas and other organs of rats and, after growth had taken place, they centrifuged the malignant and the normal cells together. This ingenious method did not provide that direct comparison of malignant cells with their normal prototypes which is necessary to bring out the actual differences, because the malignant cells did not originate in these organs. Guyer and Claus reported that the resistance to displacement of the contents of tumor cells of various types is different. It is

* Aided by grants from the U. S. Public Health Service, and from an anonymous donor.

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METHODS

Like Guyer and Claus, we used an ultracentrifuge of the Beams's type. The internal diameter of our rotor was 2.3 cm. and its depth 0.55 cm. Whereas Guyer and Claus used compressed air as the driving force, we employed oxygen at a pressure of 60 lbs. per square inch. This gave us about 120,000 r. p. m.—a displacing force in the neighborhood of 350,000 times gravity. Guyer and Claus calculated their force to be 400,000 times gravity. We centrifuged for 30 minutes after attaining the speed mentioned while they centrifuged for 1 hour. In our experiments, as in theirs, the tissues were centrifuged in Locke's solution.

Conditions were standardized as well as possible. The rotor always felt distinctly cooler immediately after rotation by oxygen pressure than at the beginning. This decrease in temperature seemed to be uniform. In one instance room temperature was 26.8° C., and it was assumed that the Locke's solution in the rotor had become stabilized at the same figure. After centrifugation at the speed mentioned for 30 minutes, the temperature of the fluid in the rotor was taken by a thermocouple of constantan + copper wire, calibrated on a Leeds and Northrop type R galvanometer, using ice water and water at known temperatures from 8° to 25° C., and found to be 16.9° C.—a decrease of 9.9° C. When the ultracentrifuge was driven by compressed air at approximately the same speed for the same time, the temperature rose from 28.5° C. in the room to 32.0° C. in the Locke's solution in the rotor—an increase of 3.5° C. The decrease in temperature in our experiments in which compressed oxygen was uniformly employed would tend to increase viscosity and decrease the rate of autolytic changes.

The time intervening between excision of living tissue and its placement in the rotor was not always uniform. The transfer was made very quickly in the case of mouse tissues, the interval being probably less than 1 minute. For excised human tissues the interval was longer; but not sufficiently so, we think, to modify the results. Only in the case of human fetuses was the interval long enough to modify the results. On delivery, the fetuses were wrapped in gauze moistened with physiological salt solution and placed in an ice box at about 2.2° C. until they

the embryos, newborn and normal adults, were treated with 0.6 per cent methylcholanthrene (Eastman Kodak Co.) in benzene. This was applied uniformly to the backs of the mice three times a week with a micropipette. Treatment was continued through-

TABLE I
Material

Tissue	Mice	Human
Adult epidermis	4, 8 and 12* months old	Female Negro, age 45; thigh Female Negro, age 48; abdomen Male, age 52; back
Hyperplastic epidermis	Treatment with methylcholanthrene for 11, 49, 51 and 64 days	From healing mastectomy wound in female, age 53
Papillomata	Treatment for 70 and 80 days	Warts from great toe of male, age 12, present 6 months; and from second finger of female, age 42, present 3 months
Squamous cell carcinomata	Treatment for 85, 99, 116, 130,* 140, 160* and 165* days	One each from helix of ear, posterior aspect of neck, cervical region, cheek, external auditory meatus, anterior auricular area, and two from lip
Transplantable tumors	One rapidly and one slowly growing MMS-21 sarcoma† Two rapidly and two slowly growing mammary carcinomas, 2163†	
Epidermis of newborn	12 hours old	Premature infant 7¾ months' gestation, lived 10 hours
Embryonic epidermis	18 days old	Two embryos of 12 weeks; one each of 16, 27, 31 and 40 weeks

* Roscoe Jackson Memorial Laboratory strain "A."

† Dobrovolskaia-Zavadskaia strain III.

out the periods mentioned, even after the appearance of grossly recognizable lesions.

2. A few strain "A" mice were treated in the same way.

3. Mice of the Dobrovolskaia-Zavadskaia strain III, bearing transplantable tumors also were used.

centrifuged, a peculiar condition was observed. Large cytoplasmic vacuoles appeared, chiefly in the spinous cells, rarely in the basal cells and never in the corneal cells. These vacuoles were characteristically spherical and usually there was but one per cell. They compressed the nuclei into crescent-shaped structures. The direction of nuclear compression was irregular and did not always correspond with that of the centrifugal force. In some portions of a given epidermis vacuoles and deformed nuclei were quite common, while in nearby parts they were absent.

When two samples of skin from the thigh of a normal white male 45 years old were placed, one with the dermis toward the periphery, the other reversed with the epidermis next the periphery, and centrifuged together, the vacuolation and resulting nuclear deformations were restricted to the first specimen. No explanation of this difference is offered. The phenomenon was never seen to the same degree in other tissues. It was occasionally manifested by a few cells in the epidermis of newborn mice. It was still less frequent in epidermis bordering papillomata and carcinomata but more often in human skin than in that of mice. Only in very rare cases was it observed in malignant cells of mice and of human tissues, accompanied by evidences of marked edema or of local necrosis.

For routine purposes a single histological technic was employed throughout. The fixative was Bouin's fluid. All paraffin blocks were cut in short strips of serial sections 5 μ thick. These were mounted and stained with hematoxylin and eosin. We intentionally used this relatively simple method because the results are fairly constant and the colors do not fade quickly, although a sharper contrast between basophilic (basi-) chromatin and acidophilic (oxy-) chromatin could have been secured by more selective staining.

To avoid misunderstanding it is desirable to characterize the terms we have used. Bodies in epidermal cells termed *nucleoli* usually stain with hematoxylin but they may possess a core of acidophilic material. They are more like amphinucleoli than true nucleoli or plasmosomes. They exist in living cells in approximately the same form and position. *Chromatin* is likewise stained with hematoxylin, often more deeply. It is seen as a cloud, in fairly discrete particles which grade in size up to false nucleoli

were sent to us. Several were useless. We got the impression that resistance of nuclear contents to displacement increases with the degree of postmortem change. This was demonstrated experimentally as follows:

1. Two newborn rats were selected. Skin from the back of the first was immediately centrifuged while the second was wrapped in gauze, moistened with saline and kept in a closed glass vessel in an ice box at 1.2° C. for 20 hrs., after which skin was removed and centrifuged. Nuclei of spinous cells of both showed the same degree of displacement, but in the second the nuclei of the basal cells and of the roots of hair follicles in a few areas were not displaced as in the first and showed the typical postmortem appearance.

2. Three mouse embryos (age 18 days) were studied. The skin from 1 was centrifuged at once while the other 2 were kept in the same ice box for 36 hrs. The excised skin from the latter 2 responded differently: in 1 the displacement was similar to that in the fresh specimen and numerous mitotic figures were seen, while in the other some nuclei showed postmortem change and resistance to displacement. This difference suggests the operation of some other factor than length of time, possibly drying.

3. In a human fetus of 8 weeks, kept similarly in the ice box for 70 hrs., most of the epidermal cells were lost and the few that remained contained shrunken nuclei, the contents of which were not displaced.

It was found that perhaps the most important factor preventing displacement is drying of the tissue. If evaporation is permitted from the surface not protected by a keratinized layer, the nuclei of the superficial cells shrink, stain more homogeneously and appear to tighten up, for their contents become very resistant to displacement.

An effort was made to reduce all specimens to a uniform size and shape before placing them in the rotor. The dimensions were usually about 3 by 3 by 2 mm. The tissues were flattened a little against the periphery of the chamber of the rotor by the centrifugal force which decreases as the axis of the rotor is approached from the periphery. Calculations show that cells 1 mm. distant from the periphery (nearer the center) are subjected to a reduction of 4.34 per cent in force as compared with those at the periphery and that the force for the cells 2 mm. distant is 8.7 per cent less. The cells that we studied were almost invariably within the most peripheral millimeter.

The manner of orientation of the tissue in the rotor is important in some cases. In a piece of human skin, which was placed in the rotor with the dermis toward the periphery and

close to the hair follicles, the nucleoli were slightly displaced centrifugally. At the same time a shift of chromatin often occurred in the same direction. Mitotic figures were unchanged. There was no displacement of the contents of pyknotic and shrunken nuclei of the corneum. On the whole, the displacement was less in normal centrifuged epidermis than in any of the epidermal lesions of mice.

Hyperplastic Epidermis

Treatment with methylcholanthrene causes hyperplasia and thickening of the epidermis. In the normal epidermis of the mouse, one can distinguish only the inner cells and outer keratinized ones, while strata similar to those usually present in thick human epidermis were found in hyperplastic mouse epidermis.

After treatment for a period of 11 days only very slight signs of displacement of the nuclear contents of basal cells were noted. Many showed none. When present, displacement was more marked in basal cells near hair follicles. Chromosomes were not displaced. Nuclei of spinous cells are larger, more spherical, and less rich in chromatin than those of the basal cells. In them displacement was more marked (Fig. 2). Occasionally spinous cells were seen, the centrifugal cytoplasm of which stained more strongly than the centripetal cytoplasm, indicating, perhaps, a concentration of substance. In the corneum there was no displacement of the contents of pyknotic, shrunken nuclei.

Specimens centrifuged after treatment for 49, 51, and 64 days exhibited similar displacements of nuclear contents, though they were less marked. In some cells of the granular layer the nucleoli and chromatin were displaced while the cytoplasmic granules retained their positions unaltered (Fig. 3). In all specimens of hyperplastic epidermis the displacement was of patchy distribution. Areas were encountered in which none could be detected; but displacement was in general distinctly greater than in normal untreated epidermis.

From hyperplastic epidermis, a papilloma may develop which may or may not become malignant. From it, also, may develop squamous cell carcinoma.

(net knots, basophilic nucleoli) and in amorphous masses, some of which cling to the nucleolus and to the nuclear membrane. We include under the same term, without any implication as to their nature, strands of material (reticulum, linin) which may be stained less strongly with hematoxylin or may even take a little eosin. The exact distribution of such chromatin in the living cell is not known, since it cannot be seen microscopically. It is safe to assume that part of the aggregation into masses is due to the action of the fixative. In considering its displacement, we must bear in mind that we are probably dealing with a fluid, partly made up of thymonucleic acid, which is present more diffusely in the nucleoplasm before centrifugation than when studied in stained preparations of normal cells. The *nucleoplasm* is often incorrectly referred to as nuclear sap because the word "sap" implies a fluid material like the sap of plants. This term should not be applied to a gel, which is sometimes the condition of the nucleoplasm.

Feulgen's reaction for thymonucleic acid was used in special cases according to the method described by Cowdry (1928). No preparations were made of the elusive cytoplasmic material, known as the Golgi apparatus, to which Guyer and Claus devoted considerable attention. Preparations were only made of mitochondria to gain information whether certain cells were degenerating.

OBSERVATIONS

The order of presentation is first to describe the displaceability of nucleoli and chromatin in normal epidermal cells and in our carcinogenic series of mice. Then an attempt is made to correlate displaceability with rapidity of growth of transplantable tumors. Finally the epidermis of newborn and embryonic mice is studied. The less adequate human material is described in substantially the same order. The tissues of mice (Figs. 1-16) and of human beings (Figs. 17-24) are briefly compared before the discussion of results.

TISSUES OF MICE

Normal Epidermis

After centrifugation (Fig. 1) there was no uniform displacement of nuclear contents. However, in small areas, especially

It would appear to be a simple matter to trace the changes in degree of nuclear displacement when hyperplastic cells become malignant, but it is not so. There is no known microscopic evidence of malignancy observable in single cells by which its onset can definitely be dated (Cowdry, 1946). Malignancy can only be defined in terms of behavior and it is possible that cells may possess the power of invading other tissues before they actually break loose from their parent tissue and become able to exhibit it. However, since nucleoli and chromatin can be displaced by centrifugal force with considerable regularity both in hyperplastic epidermal cells and in the cells of a squamous cell carcinoma, their displaceability is not a sudden change which marks the assumption of behavior that we call malignant.

The degree of displacement is not uniform throughout a squamous cell carcinoma. None of the carcinomata studied in this series was large or of long standing. Consequently there was but little central necrosis. In all of them, however, there were a few scattered cells, often with acidophilic cytoplasm and small, or distorted and pyknotic, nuclei. These cells were evidently dead, or dying, at the time of fixation. Their nuclear contents were not displaced. Occasionally, mainly in low-grade carcinomata, groups of cells were seen which were rather small in size and fairly uniform in shape. Their nuclei contained finely granular, dust-like chromatin and one or two small nucleoli. In some cases, these cells made up the tips of processes extending into the dermis. In others, they were more deeply placed and separated from the epidermis. Their nuclear contents resisted displacement.

Displacement was greatest and most uniform in cells of variable size, shape, and staining reaction, characteristic of grade III or grade IV squamous cell carcinomata. Thus, displacement is seen in almost every active malignant cell included in Figures 8 and 9. The level of separation between the displaced chromatin and the clear nucleoplasm looks a little like the surface of a fluid. It is more definite than in either the hyperplastic spinous cells of the epidermis (Fig. 2) or in the hyperplastic cells of the proximal part of a papilloma (Fig. 5). These malignant cells, before centrifugation, were richer in chromatin than the non-malignant hyperplastic cells. This may have contributed to the formation of a more conspicuous separation level.

Papillomata

The distal and better differentiated portion is made up of papillae which have grown outward. Figure 4 illustrates a section through a papilla cut in a plane vertical to the skin surface and parallel to its long axis. In the center is the vascularized core and on either side are basal cells which have given rise to spinous cells on the right and to keratinized cells on the left. Displacement of nuclear contents was very slight in the basal cells, more in the spinous cells and absent in the keratinized cells. Chromosomes were not displaced. This portion of the papilloma was obviously not in the line of carcinogenesis.

The proximal part, in which the cells are less well differentiated, consists of thickened and elongated processes which project into the dermis. A small area is represented in Figure 5. Comparison with Figure 4 shows that the cells and their nuclei are larger and the displacement of nuclear contents by centrifugal action was more marked. Other areas exhibit numerous mitoses and but little evidence of keratinization. In some places the chromosomes were shifted centrifugally. In several respects, therefore, the proximal parts of the papillomata are less differentiated than the hyperplastic epidermis which does keratinize, though the displacement of nuclear contents may be of about the same order—in some, a little more, and in others, a little less. The cells are not necessarily committed to become malignant, because even this proximal part of a papilloma does not always give rise to a carcinoma.

Squamous Cell Carcinomata

We deal with the origin of carcinoma from hyperplastic epidermal cells of the general skin surface but do not exclude the possibility that it may arise from the hyperplastic cells of hair follicles. The problem of the participation of hair follicles in carcinogenesis is left for consideration elsewhere.

Figure 6 shows, at low magnification, an area of hyperplastic epidermis on the left grading into a carcinomatous area on the right. Figure 7 illustrates, at higher magnification, a portion of the hyperplastic epidermis in which almost all the nuclei show displacement of nuclear contents. The tissue was treated with methylcholanthrene for 160 days.

centage of their nuclei showing displacement of contents when subjected to equal centrifugal force.

Three pairs of mice* were used, with one in each pair bearing a rapidly growing tumor and the other a slowly growing example of the same tumor. The neoplasms were a sarcoma and mammary adenocarcinomata. Pieces of the rapidly and slowly growing tumors in each pair were centrifuged together.

At first sight, the results were unexpected and not in line with the observations on squamous cell carcinoma, for but little difference was noted in the incidence of nuclei with displaced contents in the rapidly and slowly growing sarcomata and mammary adenocarcinomata. Indeed it appeared that displacement was more evident in the slowly growing ones.

However, the comparison was not all that could be desired. Though the sarcoma grew rapidly in one mouse and slowly in its mate, it was not a case of two kinds of tumor cells possessed of different growth rates. On the contrary, the sarcomatous cells were of the same kind in both animals and therefore might be supposed to have nuclei the contents of which possessed similar displaceability under centrifugal force. How definitely fixed is this property in any type of malignant cell remains to be determined. It is possible that the difference in rate of growth of these sarcomata was conditioned by some difference in the hosts.

The same can be said for the carcinomata in the two pairs of mice. Again, it was a case of the same type of malignant cell growing rapidly in one mouse and slowly in its mate. This experiment does not, therefore, afford an answer to the question. We hope at a later date to compare the displaceability of nuclear contents of two different transplantable tumors, one endowed with rapid growth potential and the other with slow growth, subjected to equal centrifugal force.

Epidermis of Newborn Mice

The experiments were then extended to the epidermal cells of younger mice with the idea that these might in some respects resemble malignant cells more closely than the epidermal cells of adults already described.

* These animals were supplied by William H. Woglom of Columbia University.

Figure 10 shows malignant cells fixed in the act of invading muscle. A large and a small muscle fiber extend from above downward and to the right. Both large and small nuclei have displaced contents, which are more evident in the large ones because there is more material to displace and a longer distance for it to move through.

In such active parts of a carcinoma, mitoses were quite numerous. The chromosome clumps were more uniformly and extensively displaced than in the cells of hyperplastic epidermis or papillomata (Figs. 11-13). In Figures 12 and 13, stainable cytoplasm is also represented as displaced. Evidence that the cells were active is given by mitochondrial preparations, for the mitochondria in nearby cells of the same general appearance were of the form, number, and distribution that one would expect to find in active, malignant cells.

TABLE II
Grade of Malignancy of Mouse Tumors and Percentage of Nuclei with Contents Displaced

Treatment	Grade	Percentage displaced
85 days, back	II	84.3
99 days, back	II	88.5
160 days, ear	IV	91.2
160 days, back	IV	97.1
165 days, back	III	98.0
165 days, back	II	91.9

Six squamous cell carcinomata were chosen for a preliminary quantitative study. In Table II, the duration of treatment with methylcholanthrene and the location of the tissue are given in the first column; the grade of malignancy, in the second; and the percentage of nuclei with displaced contents of 1000 examined in a typical area, in the last. Though much depends on the parts of the sections selected, and the numbers of nuclei examined were not large, there was a general correlation between grade of malignancy and percentage of displacement.

Transplantable Tumors

Efforts were made to discover the relation, if any, between the rapidity of growth of transplantable tumors and the per-

Hyperplastic Epidermis

In the epidermal cells at the edge of a healing wound, slight displacement of nuclear contents of about the same degree as in hyperplastic mouse epidermis followed centrifugation.

Displacement in hyperplastic epidermis is represented in Figure 18 from an area near a squamous cell carcinoma (Fig. 19). The contents of the nuclei of basal cells are but slightly displaced, if at all. In the spinous cells the shape of the nuclei is conspicuously changed. They look like crescents in clear vacuoles. These vacuoles are of approximately the same size as the spinous cell nuclei in uncentrifuged hyperplastic epidermis. It seems that, while the convexity of the centrifugal sides of the nuclei is maintained, the centripetal sides have been forced into the nuclear substance with loss of fluid from the nucleoplasm in a centripetal direction. From an original spherical shape the nuclei have become shallow cups and the volume of nucleoplasm has been sharply reduced. Some of the nuclei of spinous cells in hyperplastic mouse epidermis show similar but less extensive shrinkage on centrifugation. This is illustrated in Figure 2, which, however, was taken at a higher magnification than Figure 18. There is, therefore, a similarity in the effect of centrifugal force on human and mouse hyperplastic epidermis. This kind of nuclear deformation is not found in active carcinomatous cells.

Papillomata

Displacement of nuclear contents following centrifugation was slight and limited to the nuclei of a few spinous cells in a large wart removed from the big toe of a boy 12 years old; but no displacement of nuclear contents was observed in a smaller wart excised from the hand of an adult female. The resistance to displacement was a little greater than in the well differentiated, distal parts of papillomata of mice.

Squamous Cell Carcinomata

Figure 19 shows at low magnification the general character of the lesion from which Figures 20 and 21 were taken. A portion of an epithelial pearl is illustrated in Figure 20. The nuclei exhibit some displacement of nucleoli and chromatin. A nucleus below and to the left is shrunken and partially surrounded by

Figures 14 and 15 illustrate the centrifuged epidermis of a newborn mouse. The first is from a region between hair follicles and should be compared with Figure 1 of the adult epidermis. The nuclei are not rich in chromatin, as in the adult, but displacement of nucleoli is more marked. In Figure 15, nearer to a hair follicle, there are more layers of nuclei which exhibit some displacement. The mouse had been born only 12 hours and the peripheral cells had obviously undergone quick desiccation and rapid keratinization. The keratinized layers were noticeably thicker than in the adult (Fig. 1). Mitoses were infrequent.

Embryonic Epidermis

In the epidermis of a mouse embryo of 18 days, which was centrifuged for only one half the regular time, the displacement of nuclear contents was very extensive (Fig. 16). The surface of this epidermis had not dried and the nuclei of the most superficial cells showed as marked displacement as the basal cells. The level of separation between displaced chromatin and clear nucleoplasm is even more definite than in malignant cells (Figs. 8 and 9). The uniformity of displacement, as of the size of the nuclei, is greater than in the carcinomata.

It is interesting to note that the shape of these embryonic nuclei was little, if at all, distorted by centrifugation. They were not drawn out into long strands with bulbous ends, like those observed in the tissues of embryonic chicks by Beams and King (1936) who used less force for a shorter time.

HUMAN TISSUES

Normal Epidermis

The nuclei of normal epidermis, as in mice, were very resistant to centrifugation (Fig. 17). In some places, especially near the tips of the rete pegs deep in the dermis, slight displacement of chromatin was detected in both basal and spinous cells. This, however, was not uniform. It was of patchy distribution and more often absent than present. In no case were chromosome clumps displaced. Nuclear displacement was not seen in the so-called clear cells near the basement membrane.

of spinous cells. The remainder of the epidermis had evidently suffered some desiccation.

Embryonic Epidermis

Pieces of skin from six fetuses (Table I) were centrifuged for 30 minutes. In all but one of them, however, the resulting specimens were useless because the tissue had been injured mechanically, allowed to autolyze or to dry. The single fetus that was of use for our purpose was of 4 months' gestation. It was dead when spontaneous abortion occurred and tissues were taken 55 minutes after delivery. Five pieces of skin from the back were immediately centrifuged for 30 minutes in Locke's solution. Five others were placed at the same time in Locke's solution for 30 minutes. Of these, two pieces were fixed in Bouin's fluid to serve as uncentrifuged controls and the remainder were centrifuged.

All of the centrifuged specimens showed considerable variability. In all of them some nuclei, usually in clumps, exhibited the shrunken, pyknotic appearance characteristic of both post-mortem changes and drying. But in some there were fairly large stretches of epidermis in which the nuclei looked normal. In such regions two modifications were noted.

First, there was slight displacement of nucleoli and chromatin of epidermal cells and of the cells at the roots of hair follicles, but this displacement was of patchy distribution and many normal looking nuclei did not show it. In no case did the displacement even remotely approximate the stratification of nuclear contents in the embryonic cells of mice (Fig. 16).

The second and most consistent finding related to the position of nuclei in the epidermal cells. The direction of centrifugal force was from the interior toward the surface. Long rows of cells were observed with their nuclei located in the distal cytoplasm leaving the proximal cytoplasm clear. Nuclear shape, however, was not altered. In the uncentrifuged control specimen, a considerable number of nuclei were similarly placed but their distal position was in general less definite. Perhaps the centrifugal force enhanced a shift in position of the nuclei. Whether this peculiar location of epidermal cell nuclei is normal or results in some way from the many small injuries sustained by this tissue before examination, further work alone will show.

a large vacuole of approximately the same dimensions as was the nucleus before it lost fluid. The persistence of the original nuclear outline in the cytoplasm suggests that the cytoplasm is of firmer consistency. In parts of this carcinoma, where the cells are very actively invasive and show a marked nuclear hyperchromatism, there is an accompanying marked displacement of nuclear contents (Fig. 21).

Figure 22 is a photomicrograph at low magnification of a highly malignant squamous cell carcinoma. Hyperplastic epidermis is included below and to the right. Figures 23 and 24 are from the same tumor. The first shows the uniformity of nuclear displacement in hyperchromatic nuclei of a considerable range in size. As in the mouse tumor (Figs. 8 and 9), so also here the line of separation between chromatin and clear nucleoplasm is fairly even and implies a fluid level. The second (Fig. 24) is at a much greater magnification and shows that in certain nuclei strands of stainable substance stretch across the clear nucleoplasm in the direction of displacement of chromatin.

A quantitative study of squamous cell carcinomata was made by the same methods used for mouse tumors to show the percentage of nuclei exhibiting displacement. One thousand nuclei were examined in active areas where there were many mitotic figures. The results are presented in Table III, which should be compared with Table II for mice.

TABLE III
*Grade of Malignancy of Human Tumors and Percentage of Nuclei with Contents Displaced**

Location	Grade	Percentage displaced
1. Ear	III	89.3
2. Lip	II	88.5
3. Neck	II	85.5
4. Lip	II	81.2
5. Lip	II	70.4
6. Check	II	80.7

* A basal cell carcinoma showed 98.7 per cent of its nuclei with contents displaced.

Epidermis of the Newborn

Skin from a premature baby (7¾ months' gestation) that lived 10 hours was centrifuged in the usual way. The resulting specimen showed only very slight displacement in a small area

with most water grow the fastest. The same relation holds, to some extent, for normal tissues, since the tissues of embryos generally have a higher water content and grow faster than those of older individuals. Exceptions were enumerated by Needham (1931) and exceptions may likewise occur for malignant tissues. It is not known how the extracellular spaces, the cytoplasm and the nucleoplasm share in housing this relatively larger amount of water. Perhaps in both the malignant and embryonic cells which we have studied, the nuclei have a greater water content per unit of volume than those of normal adult cells, which might result in a lower specific gravity of their nucleoplasm.

Our finding of the greater displaceability of nucleoli and chromatin in malignant cells than in their normal prototypes might conceivably be due, therefore, either to an increase in the specific gravities of these components in the malignant cells as compared with normal ones or to a decrease in the specific gravity of the nucleoplasm, while the specific gravities of the components mentioned remained the same.

VISCOSITY

According to Heilbrunn (1937): "Viscosity can be roughly defined as the force which tends to hold the particles of a substance together when a shearing force acting on the substance tends to pull it apart." Viscosity, he says, is the inverse of fluidity.

To identify all the factors that may condition intranuclear viscosity is, of course, quite beyond us. Reference has been made to the likelihood of a change in the water content. But an alteration in the distribution of the same amount of water within a nucleus may also be important, as when a hydrosol gels. Utilizing the cessation of Brownian movement of particles in the nucleus viewed in the dark field as an indication of gelation, Lewis (1923) found that gelation of nuclear contents can be produced in living cells by weak acids and that return to the sol phase can be effected by washing with dilute alkalis, all without killing the cells.

It is probable that a swing of the reaction toward the acid side in nuclei of the dying or dead cells of those tissues, which in our experiments were intentionally kept for some time after excision

DISCUSSION

Though we have found greater displacement of nuclear contents in malignant cells than in normal cells of the same sort, while Guyer and Claus observed less displacement in the tumor cells which they studied than in certain other normal cells, our findings are not incompatible with theirs because the tissues examined were different. Their observations relate to the entire cell substance and ours only to the nucleus. They assume that degree of displacement is conditioned by degree of viscosity and reject the possibility that a change in specific gravity of cellular components may be involved.

SPECIFIC GRAVITY

That substances of greater specific gravity are displaced more than those of lesser specific gravity is to be expected. Beams and King (1936) have reported the stratification of nuclear contents of embryonic chick cells "in the order of their decreasing specific gravity as follows: (1) nucleoli, (2) chromatin and reticulum and (3) nuclear sap." Ultracentrifugation has also been used to determine the weights of intranuclear inclusions caused by viruses relative to other nuclear components. Thus, Lucas and Herrmann (1935) have concluded that herpetic intranuclear inclusions are lighter than either the nuclear sap or the chromatin; while Rosenbusch and Lucas (1939) found that the intranuclear inclusions in the salivary glands of guinea pigs are heavier than the nuclear sap. But these observations are concerned with the relative displacement of nuclear components within the same nucleus. Our problem is to measure and explain the difference in displaceability between different nuclei.

We have been unable to discover any direct evidence either for or against a change in the specific gravity of nuclear materials in the transformation of normal cells into malignant ones. However, the nucleoli of malignant cells are sometimes slightly different both in size, number and appearance from those of their normal prototypes, and the amount of chromatin may be different. It is also possible that the water content of the nucleoplasm may not be the same. Cramer (1916) has reported, and it has since been confirmed, that the water content of various transplantable tumors varies directly with the rate of growth. Those

tiated, is not surprising. It is, however, interesting that the nuclei of spinous cells often show more displacement than the nuclei of nearby basal cells. This suggests a decrease in viscosity with differentiation of the spinous cells from basal ones. The nuclei of spinous cells are regularly larger and often more spherical than those of the basal cells. They contain less chromatin per unit of volume. Both of these differences are consistent with the assumption that their water content is greater. In their comparatively large nucleoli and small chromatin content they approach the nuclei of nerve cells and of ova, the contents of which are easily displaced.

It would seem that intranuclear viscosity, low in embryonic epidermal cells, rises in basal cells, falls in spinous cells, and rises again as the cells die because in the most superficial cells there is no displacement of nuclear contents. Similarly in the carcinogenic series it is low in embryonic epidermal cells, high in normal basal cells, lower in spinous cells and lower still in malignant cells, only to rise again as the malignant cells die.

Low intranuclear viscosity is not a characteristic of malignant cells which normal cells of the same type are unable to exhibit. But there are differences. The malignant cells which show it may be as rich in chromatin as embryonic cells of the same type, whereas the spinous cells contain relatively only a little chromatin. Moreover, the malignant cells may contain, proportionately to their size, as much chromatin as the basal cells of the epidermis in which the nuclear contents resist displacement. It is in the association of high chromatin content with low viscosity that the nuclei of malignant cells resemble those of the embryo. But in the descendants of malignant cells this property of low viscosity is fixed in so far that it reappears after each cell division, whereas it usually decreases during normal development.

We have made no attempt to measure the speed of these nuclear changes evidenced by groups of cells. The marked increase in viscosity between the embryonic and the newborn state of the epidermis of mice may be fairly abrupt owing perhaps to rapid desiccation of the superficial layer of cells. The slight decrease from normal epidermis through the higher grades of hyperplasia is probably much slower. The decrease in intranuclear viscosity with the formation of squamous cell carcinoma is very consider-

and before centrifuging, caused a gelation and so increased the density of their nuclear contents as to prevent displacement. Orr (1937) found evidence of increase in acidity during carcinogenesis in skin lesions of mice treated with dibenzanthracene and other substances by using phenol red as an indicator *in vivo*. If this acidity were to produce nuclear gelation, as the other acids did in the experiments of Lewis, we would expect an increase in intranuclear viscosity and a decrease in displaceability of nuclear contents under centrifugal force, which is the converse of what we have described. But the acidity may be of tissue fluids and may not extend to the cytoplasm or nucleoplasm. Results obtained by Rous (1925) and by Drury and Rous (1926) are compatible with this localization. Moreover, Chambers and Ludford (1932) injected indicators directly into the cytoplasm of malignant and normal cells in tissue cultures and could discover no significant difference in P_H .

While, therefore, admitting the possibility that a change in specific gravity of nuclear components may occur in the formation of malignant cells from normal ones and that this may partly condition the difference in displaceability, we think that it is safe to conclude that the main factor is a change in intranuclear viscosity and that in our series, easy displaceability of nuclear contents is a reliable indicator of low viscosity, and greater resistance to displacement an indicator of higher viscosity. In this qualified conclusion, we agree with Guyer and Claus.

Though practically all of the nuclei of the epidermis of the mouse embryo showed displacement, and almost all of the nuclei of active cells of squamous cell carcinoma also exhibited displacement, the phenomenon is less common in hyperplastic cells and almost negligible in the normal epidermis of adults. In the hyperplastic epidermis it is present in some areas and absent in others. Indeed, the nuclei of neighboring cells may differ markedly in their resistance to displacement. It may be that the intranuclear viscosity of normal cells of the same type and degree of differentiation is not constant, but varies with changes in physiological activity which do not find expression in structural changes detectable microscopically.

That differences in displacement of nuclear contents under equal centrifugal force are observed in cells unequally differen-

lower in the nuclei of cells rendered hyperplastic in the treated epidermis and in the proximal parts of papillomata, and *much lower* in the nuclei of squamous cell carcinomata.

Passing backward from normal adult epidermis, through the epidermis of newborn mice to embryonic epidermis, there is also a decrease in intranuclear viscosity. In their relatively very low viscosity (lack of resistance to displacement of nuclear contents), the nuclei of embryonic epidermal cells closely resemble those of squamous cell carcinomata.

In a less complete series of human tissues, similar changes were observed. Intranuclear viscosity is *high* in normal epidermal cells, *lower* in hyperplastic cells of a healing wound and in papillomata (warts), and *much lower* in the nuclei of squamous cell carcinomata.

When, with degeneration, the nuclei of normal and malignant cells of both mice and humans shrink and become pyknotic, intranuclear viscosity increases so that it is impossible to displace their nucleoli and chromatin by the centrifugal force employed.

NOTE: We wish to express our appreciation to Burton Simpson and William S. Murray for supplying the New Buffalo mice used in these experiments; to William H. Weglom for the mice of the Dobrovolskai-Zavadskai strain III; and to Gordon H. Scott for the calculations. The strain "A" mice were obtained directly from the Rostoe Jackson Memorial Laboratory, Bar Harbor, Maine.

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able and probably more than would be the case if the nuclei of the normal cells giving rise to the malignant ones had had an abnormally low intranuclear viscosity to begin with. Similarly, judging by ease of displacement of nuclear contents, the intranuclear viscosity of parenchymatous hepatic cells is relatively low. If the intranuclear viscosity of malignant cells formed from them is also low, the difference in intranuclear viscosities between this type of malignant cell and its normal prototype would not be very great. If, however, the intranuclear viscosity of this type of malignant cell happens to be high, the difference would be particularly noticeable because of the unusually low initial viscosity. It is, of course, the considerable decrease between the hyperplastic and the malignant cells, often with increase in chromatin content, that interests us most. Whether this is gradual or sudden, further work alone will show.

We have investigated only a single kind of malignant cell and its normal prototype. No generalization is justified. Data are awaited on the presence or absence of a similar decrease in nuclear viscosity in the transformation of other kinds of normal cells into malignant ones. When the skin, liver, kidney, pancreas, adrenal cortex and medulla of mice are subjected to equal centrifugal force, the displacement of nuclear contents is not uniform. It is very marked in the adrenal medulla, less in the adrenal cortex, kidney and liver and still less in the acini and islets of the pancreas and in the epidermis. Consequently it is important to note that in this study of epidermal cells and their malignant offspring, we commenced with normal cells, the nuclear contents of which are possessed of particularly high viscosity and are especially resistant to displacement.

CONCLUSIONS

When the nuclei in two pieces of tissue are subjected to equal centrifugal force, as a result of which nucleoli and chromatin are more displaced in one than in the other, there is reason to believe that intranuclear viscosity is lower in those in which the displacement is greater.

In methylcholanthrene carcinogenesis in mice, progressive changes in intranuclear viscosity occur. It is *high* in the nuclei of normal epidermal cells before the beginning of treatment,

DESCRIPTION OF PLATES

All of the photomicrographs are from sections stained with hematoxylin and eosin, prepared from tissues which had been centrifuged for 30 minutes, except Figure 15 for which the tissue was centrifuged for only 15 minutes. The directions of force are indicated by arrows.

PLATE 61

Specimens from Mice

FIG. 1. Normal epidermis. No displacement of nuclear contents. $\times 1040$.

FIG. 2. Spinous cells of hyperplastic epidermis with marked displacement of nucleoli and chromatin of spinous cells. Treatment with methylcholanthrene for 11 days. $\times 1040$.

FIG. 3. Cells of stratum granulosum of hyperplastic epidermis after treatment with methylcholanthrene for 49 days. The cell in the center shows displacement of nuclear contents without displacement of cytoplasmic granules. $\times 1040$.

FIG. 4. Distal part of a benign papilloma after 60 days of treatment. The central core of vascularized dermis is bounded on either side by epidermis. Keratinization is more advanced on the left where there is no sign of displacement of nuclear contents. On the right, however, displacement is noticeable in a few large spinous cell nuclei and barely discernible in the smaller hyperchromatic nuclei of basal cells. $\times 1040$.

FIG. 5. Proximal (deep) portion of a mouse papilloma which is less differentiated, showing displaceability in nuclear content of the cells. $\times 1040$.

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PLATE 65

Specimens from Mice

FIG. 6. Hyperplastic epidermis on the left bordering on an area of squamous cell carcinoma. Treated with methylcholanthrene for 160 days. $\times 130$.

FIG. 7. Higher magnification of hyperplastic epidermis in lower left corner of Figure 6, showing nearly uniform displacement of nuclear contents. $\times 800$.

FIG. 8. Squamous cell carcinoma. Almost every nucleus of a malignant cell shows displacement of contents. Treatment for 116 days. $\times 1040$.

FIG. 9. Squamous cell carcinoma with the nuclear contents displaced in large anaplastic cells. Treatment for 130 days. $\times 1040$.

FIG. 10. Squamous cell carcinoma with malignant cells invading muscle. Their nuclear contents are displaced. Treatment for 140 days. $\times 1040$.



Alterations in Nuclear Viscosity

PLATE 66

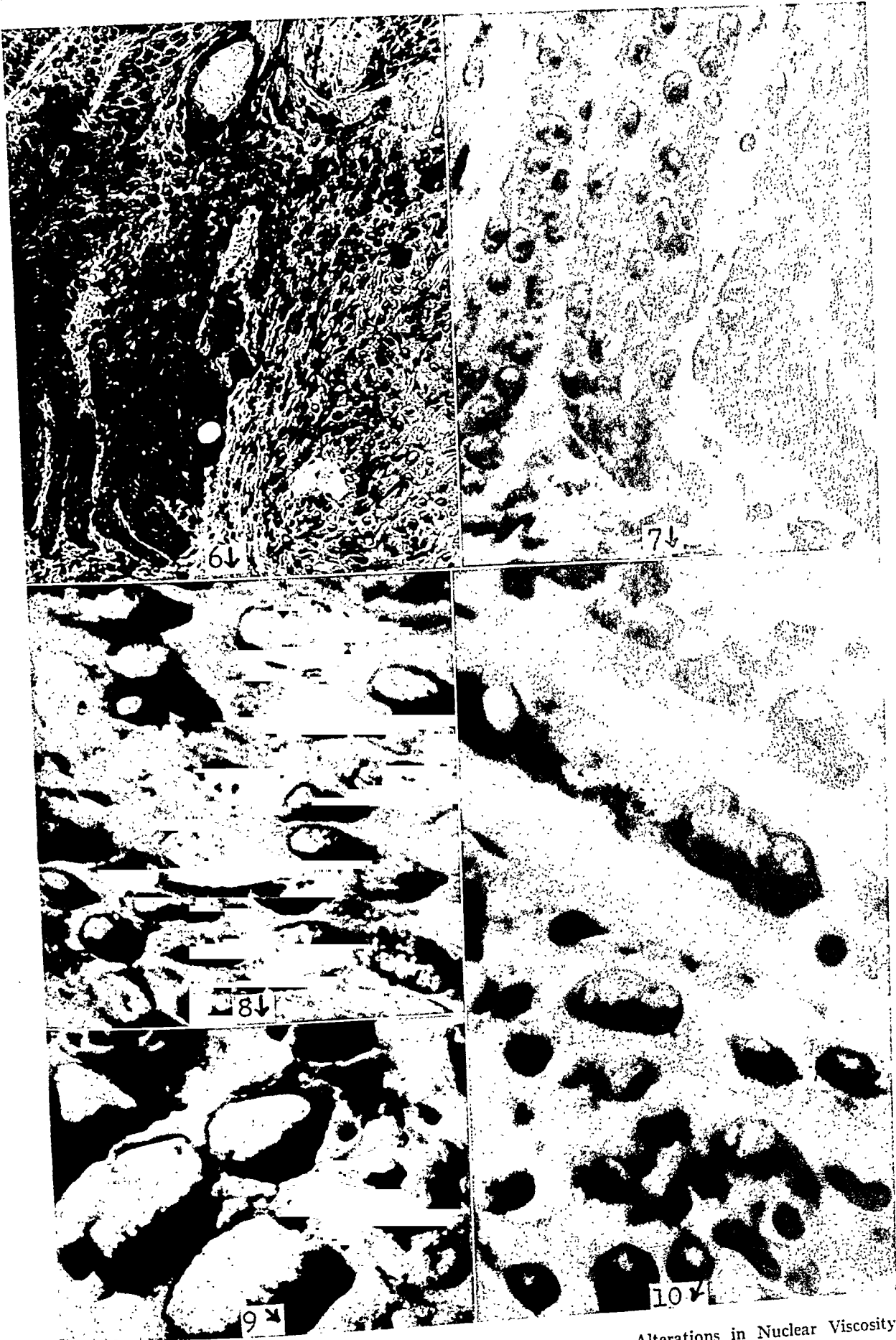
Specimens from Mice

FIGS. 11-13. Same specimen as Figure 6 but showing squamous cell carcinoma with dividing cells. There is displacement of chromosome clumps and the stainable part of the cytoplasm is also displaced in Figures 12 and 13. $\times 1040$.

FIG. 14. The untreated epidermis of a mouse, 12 hours after birth. There is marked displacement of nucleoli and of some basophilic chromatin somewhat like that found in hyperplastic spinous cells (Fig. 2), $\times 1040$.

FIG. 15. The same epidermis as in Figure 14, but near a hair follicle, also shows some displacement of nuclear contents. $\times 1040$.

FIG. 16. Epidermis of an 18-day mouse embryo with extensive displacement of nuclear contents, even of surface cells. Compare with uniformity of displacement in squamous cell carcinoma (Figs. 8, 9, and 10). $\times 1040$.



Alterations in Nuclear Viscosity

PLATE 67

Human Tissues

- FIG. 17. Normal epidermis from the back of a male 52 years old, showing very little sign of displacement. Compare with lack of displacement in normal mouse epidermis (Fig. 1). $\times 1040$.
- FIG. 18. Epidermis away from the transition point of the squamous cell carcinoma shown in Figure 19. There is very little displacement. $\times 700$.
- FIG. 19. Squamous cell carcinoma from the neck of a white male 58 years old, showing the character of the lesion. $\times 115$.
- FIG. 20. Displacement in the nuclei of an epithelial pearl. $\times 700$.
- FIG. 21. Considerable displacement in anaplastic cells invading deep tissues from the tumor shown in Figure 19. $\times 700$.

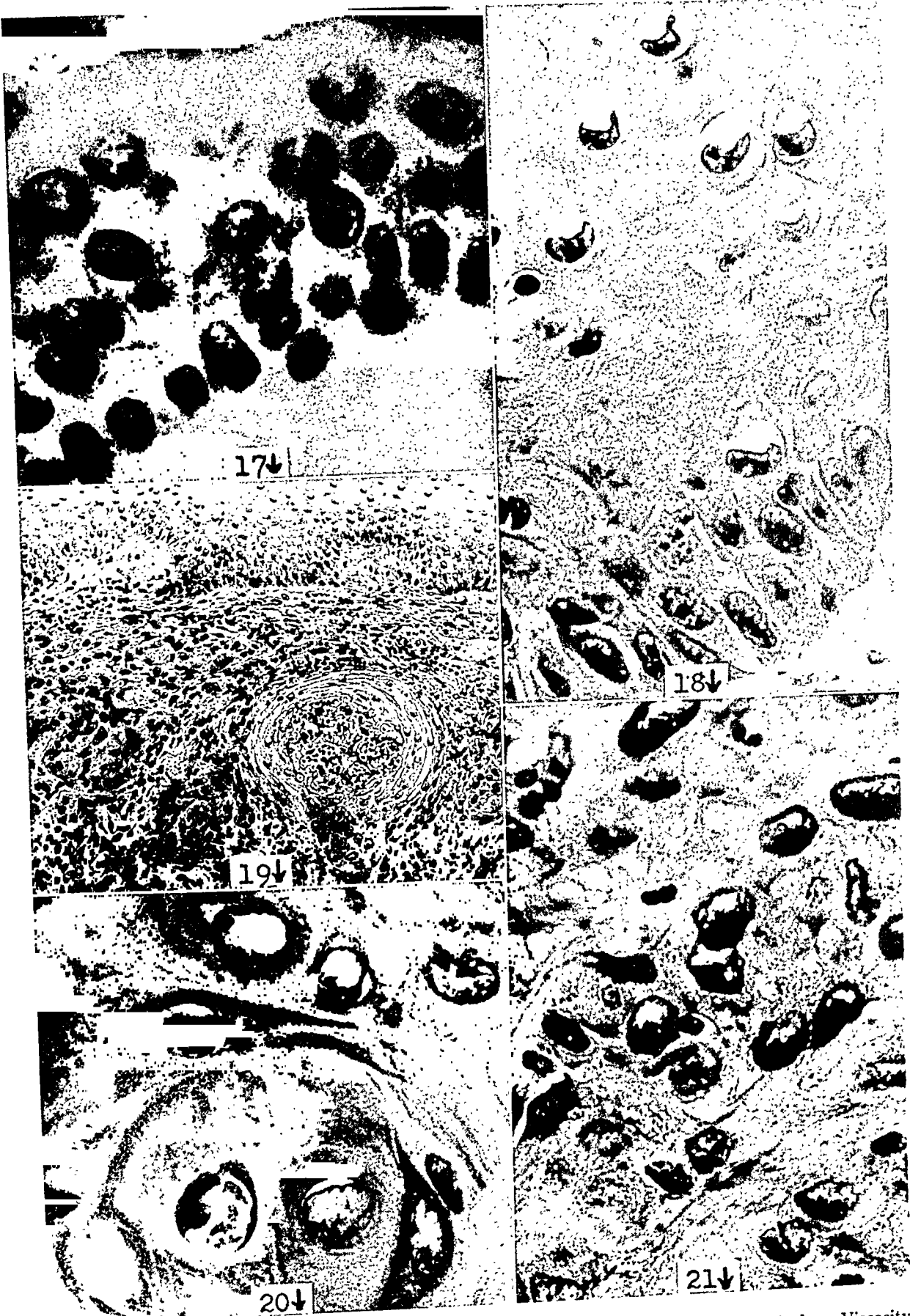


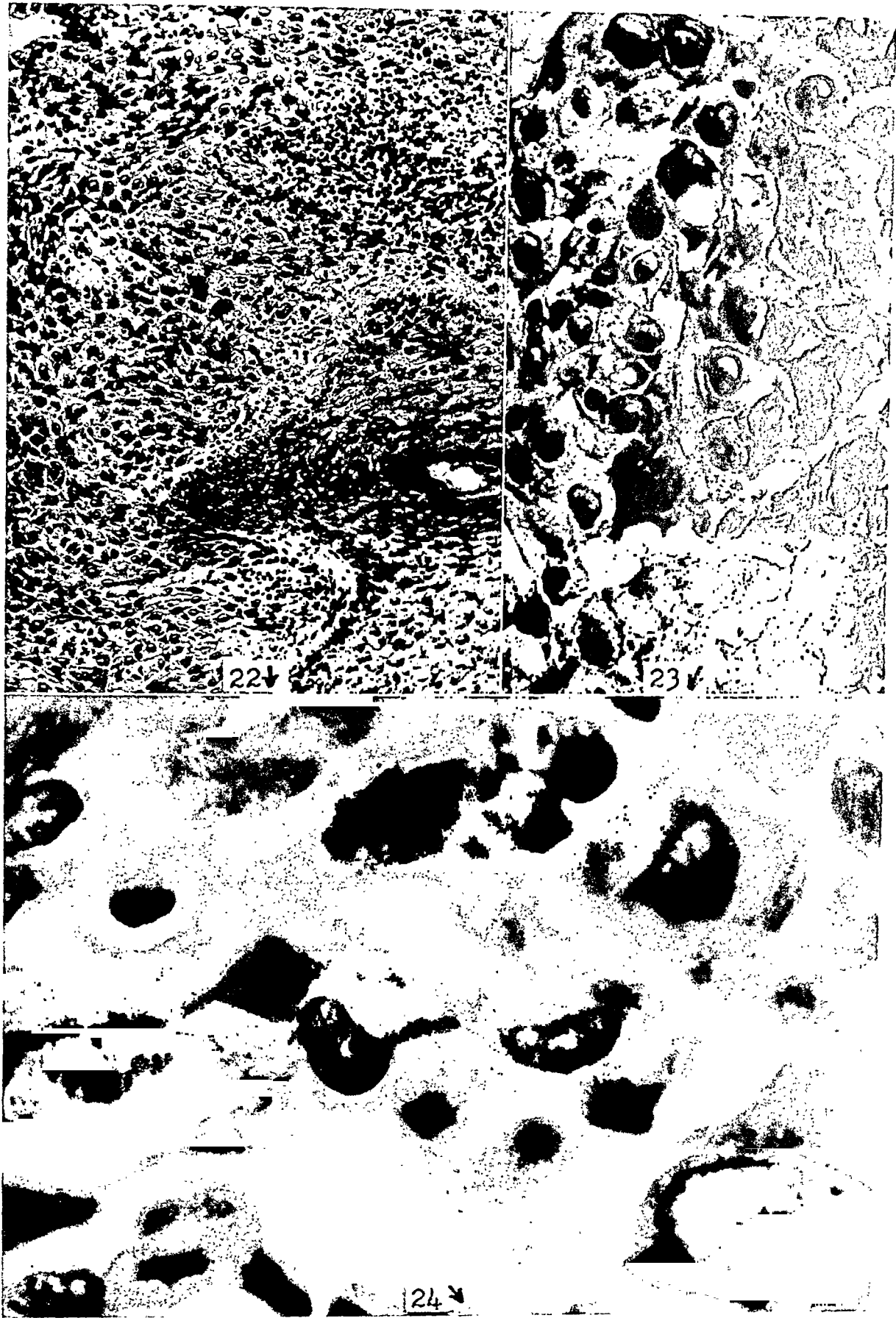
Alterations in Nuclear Viscosity

PLATE 68

Human Tissues

- FIG. 22. Squamous cell carcinoma from an ear of a white male 54 years old, highly malignant. $\times 145$.
- FIG. 23. Very anaplastic cells from the same tumor as shown in Figure 22. Every cell shows displacement of basophilic chromatin. $\times 520$.
- FIG. 24. Higher power magnification showing displacement of basophilic chromatin in highly anaplastic cells of the squamous cell carcinoma illustrated in Figure 23. $\times 1500$.





broader subject of primary inflammation of the arteries, Karsner⁶ stated: "Degenerative and mild or severe inflammatory lesions of the arteries, not directly caused by an infective agent, are well known, but few if any correlative studies have been published." It is believed that a definite correlation exists in the studies here presented.

A brief summary of the events leading up to these arterial lesions will furnish the historical background and introduce the general methods employed. In an attempt to secure a test animal comparable to the patient with nephritis for testing the efficiency for new plasma protein production of various dietary and supplementary factors, 3 normal adult dogs, maintained on a standard low protein diet, were depleted to a basal plasma protein level of 4 per cent by repeated bleeding and return of the washed red blood cells suspended in a saline solution (plasmapheresis), and the rate of plasma protein production under these standard conditions was determined. Uranium nitrate was then injected subcutaneously and the effect of the ensuing elevation of nonprotein nitrogen upon plasma protein production was followed. Despite elevations in nonprotein nitrogen to more than ten times normal, no interference with plasma protein production was observed.⁷ One unexpected finding, however, emerged from these studies: the dog with depleted plasma proteins is more susceptible to uranium nitrate injury than is the normal dog. Whereas the majority of normal adult dogs will survive the administration of 4.0 mg. of uranium nitrate per Kg.,⁸ the 2 hypoproteinemic dogs receiving 3.0 and 2.5 mg. per Kg. both died in uremia in about 2 weeks. Only when the dose was reduced to 2.0 mg., or one half the usual amount, did a dog with depleted plasma proteins survive. This observation raised the question whether increase of the plasma protein concentration would protect against heavy metal poisoning. When the procedure was reversed, *i.e.*, when the dog's plasma protein level was increased from a normal of about 6.5 per cent to 9 or 10 per cent by repeated daily injections of plasma obtained from normal healthy donor dogs, and the dose of uranium nitrate was increased from 2.0 to 5.0 mg. per Kg., the arterial lesions to be described were found in all 5 dogs on which this procedure was tried.

ACUTE NECROTIZING ARTERITIS, AORTITIS, AND AURICULITIS FOLLOWING URANIUM NITRATE INJURY IN DOGS WITH ALTERED PLASMA PROTEINS*

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In this paper are described the well marked necrotizing lesions which appeared in the aorta, sinuses of Valsalva, pulmonary artery, left auricle, and elsewhere in 5 dogs given uranium nitrate subcutaneously after being made hyperproteinemic by repeated intravenous injections of plasma obtained from healthy donor dogs. Similar lesions developed in 2 other dogs rendered hypo-proteinemic by repeated plasmaphereses before injecting the heavy metal, and in still another dog which received only two injections of donor's plasma at a considerable interval before the injection of uranium nitrate. The factors common to all of these experiments are: (1) some alteration in the plasma proteins produced by the use of "homologous" blood and (2) uranium nitrate injury. While the nature of the process has not been revealed by the experiments thus far, the lesions are striking, can easily be recognized both grossly and histologically and have been produced in the relatively short period of about 6 weeks.

Necrotizing arteritis has attracted attention chiefly because of its occurrence in periarteritis nodosa and rheumatic arteritis. Numerous workers¹⁻⁴ have postulated a peculiar allergic or hyperergic state of the blood vessels to explain the extensive necrotizing lesions sometimes seen in these diseases. The following two quotations from the rather extensive literature have been selected as pertinent: Klemperer,⁵ in discussing a case of periarteritis nodosa, stated: "I feel that we should not look for a specific bacteriologic etiology in necrosing arteritis, but should search for a specific immunologic phase or a specific sensitiveness of the blood vessels; and try to reproduce this, if possible, experimentally, instead of trying to reproduce the disease by the injection of organ extracts in such cases." While a specific allergic state has not been demonstrated in the studies here presented, there is a strong probability that such a state exists. In a review of the

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in 0.5 per cent aqueous solution. Sufficient water was used to insure quantitative transfer.

The experimental animals were kept under close observation, were sacrificed with ether if they were obviously moribund and were autopsied promptly after death. Routine sections, taken from all organs and from many of the tissues, were fixed in 4 per cent solution of formaldehyde and Zenker's solution. The Zenker's-fixed tissues were embedded in paraffin, sectioned at 7 μ , and stained routinely with hematoxylin and eosin. Special stains used in selected cases included: Verhoeff's stain for elastic tissue, sometimes combined with van Gieson's picro-acid fuchsin solution; Mallory's aniline blue stain for collagen; von Kossa's silver nitrate method for calcium; scarlet red stain for fats; and phloxine-methylene blue and Gram's stains for bacteria.

EXPERIMENTAL OBSERVATIONS

Table I gives in summary the experimental data on all of the dogs which developed arterial lesions. The anatomical distribution of these lesions is indicated in Table II. In Table I the dogs are divided into three groups and the same grouping is indicated in Table II.

Group A comprises the 5 dogs rendered hyperproteinemic by repeated daily injections (17 to 24 in the course of 3 to 4 weeks) of plasma obtained from healthy donor dogs. The dose of uranium nitrate in each was 5.0 mg. per Kg. In all but one of these dogs sodium citrate was used to prevent coagulation in obtaining the donor's plasma. Thus each daily injection, which averaged 100 cc., contained 2 to 3 cc. of saturated sodium citrate. In 1 dog (No. 40-50) heparin (50 mg. per injection) was used. The lesions in this dog were possibly a little less marked than in the dogs receiving citrate, but they were qualitatively the same.

Group C consists of 2 of 4 dogs made hypoproteinemic by almost daily withdrawals of plasma (plasmapheresis) over a period of time similar to that in Group A. In about one half of the instances, the experimental dog was bled directly and, after removing the plasma from the centrifugalized, citrated blood with the aid of suction, its own cells, resuspended in a saline solution, were reinjected. At other times, immediately after bleeding the experimental animal, the previously prepared corpuscles of a

METHODS

All of the experimental animals were normal adult dogs. The dogs used as donors were large dogs maintained on the regular kennel diet of fresh meat and table scraps. A sufficient number of these were used so that anemia did not develop. The dogs in the experimental group were carefully selected. They were slender, well nourished (but not fat) dogs of hound or terrier extraction with readily accessible neck veins and femoral arteries, and were as small as practicable so that reductions or increases of the plasma protein level could be accomplished without using too great quantities of blood. The 8 dogs in this experimental group consisted of 5 females and 3 males whose estimated age varied from 1 to 5 years. All of the dogs in this experimental group were maintained on a standard basal diet which consisted of: calves' liver (raw wet weight) 32 parts, cane sugar 25 parts, corn starch 25 parts, butter 12 parts, cod liver oil 6 parts. Enough tomato juice was added to make a paste, of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. One gm. of the McCollum-Simmonds salt mixture⁹ and 5 gm. of kaolin were thoroughly mixed with each day's diet.

The details of methods for depleting the plasma proteins to standard hypoproteinemic level have recently been published.^{7,10} The plasma injections, used in making the dogs hyperproteinemic, were carried out as follows: About 250 cc. of blood from a donor dog was bled into a flask containing 3 cc. of saturated sodium citrate and centrifugated for 30 minutes at 3500 r. p. m. in 100 cc. centrifuge tubes. The plasma was then drawn off with suction, warmed to 40 to 45° C. and injected into the jugular vein. Approximately 15 minutes was required for the plasma to run into the vein. Further details of this procedure can be found in previous publications.^{11, 12}

Duplicate micro-Kjeldahl analyses of total nitrogen, nonprotein nitrogen and albumin plus nonprotein nitrogen (the filtrate from 22 per cent sodium sulfate precipitation by Howe's method) served as the basis for calculation of plasma protein removed or injected and blood level studies.¹³

The uranium nitrate ($\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was obtained from Merck, lot S.7497, and was injected subcutaneously in the groin

uranium nitrate per Kg., developed arterial lesions; but a much larger number of animals with varying doses of uranium nitrate would be necessary before one could feel confident that this statement would continue to hold true.

Group B consists of only 1 dog which was given 2 injections totaling 125 cc. of citrated plasma obtained from donors on 2 successive days 3 weeks before the injection of 5.0 mg. of uranium

TABLE II
*Anatomical Distribution of Arterial Lesions**

Dog No.	Aorta		Sinuses of Valsalva	Coro-nary arteries	Left auricle	Pul-monary artery	Arteries in lungs	Other arteries
	Ascend-ing	Abdom-inal						
39-34	+++	++	++	+		+++	+	Mesenteric +
39-40	+++†	++	++		+++†	+++		Mesenteric +, carotid +
39-45	++	+++†				++	+	
40-50	+			+		++	+	Mitral valve +
39-28	+					+++†	+	All lesions "healed"
39-43	++			+	+++	++	+	
39-49	+		+	+	++			Mitral valve ++
39-24	+++	+++	++	++		+++	+	Innominate ++, ; lingual ++ ; fem- oral ++,† ; subclavian ++ ; inter- nal mammary ++; mesen- teric +.

* The lesions have been graded as follows: +++ = gross lesion over 1 cm. in maximum diameter; ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopic sections.

† Gross and microscopic illustration.

‡ Microscopic illustration.

|| Thrombus associated with lesion.

nitrate. Because of "nervousness" and "difficult" neck veins this dog was dropped from the hyperproteinemic group and shifted over to a control group. Since, of the 6 control dogs, this was the only one that developed lesions, it is believed that these two injections of donor's plasma are significant.

This separation of the experimental animals into groups is useful only in presenting the experimental data and in discussing the possibilities of pathogenesis, for no such separation into groups is possible from an anatomical study of the lesions.

donor, suspended in a saline solution and warmed to body temperature, were injected. Regardless of which procedure was used, after drawing off the plasma, the red blood cells were always washed once with modified Locke's solution¹⁴ before they were injected. Analysis of these first "washings" have shown that they contained only about 7 per cent as much protein as the original plasma; hence this procedure removed over 99 per cent of the plasma protein but did not remove all of this protein.¹⁴ This point is emphasized, for it is possible that the recipient is sensi-

TABLE I
*Summary of Experimental Data on Dogs with Arterial Lesions**

Group†	Dog number	Number of injections or exchanges‡	Total amount of plasma injected or removed	Plasma protein concentration		Body weight§	Uranium nitrate injected subcutaneously	Height of non-protein nitrogen	Survival interval¶
				Before alteration	After alteration				
			cc.	gm./100 cc.	gm./100 cc.	Kg.	mg./Kg.	mg./100 cc.	days
A	39-34	22	+2655	6.5	9.4	4.4	5.0	297	8
	39-40	17	+2010	7.0	8.8	6.5	5.0	582	11
	39-45	22	+2210	7.1	9.2	7.3	5.0	617	15
	40-50	24	+1880	6.6	10.0	4.8	5.0	573	17
	39-28	24	+2225	7.2	9.9	5.1	5.0	92	335
B	39-43	2	+ 125	6.6	6.1	4.7	5.0	438	15
C	39-49	17	-1492	8.2	4.0	4.8	5.0	391	15
	38-24	20	-2040	7.6	4.1	4.5	3.0	386	15

* All dogs were maintained on basal diet throughout entire period.

† Group A, hyperproteinemic; Group B, see text for discussion of this dog; Group C, hypoproteinemic.

‡ The total period in which these injections were made was 3 to 4 weeks.

§ Body weight at end of period of plasma injections or withdrawals, i.e., weight on day of uranium nitrate injection.

|| In all instances except dog No. 39-28 which survived the acute stage, the nonprotein nitrogen as recorded is on a sample taken immediately prior to death or during the previous day.

¶ Interval between injection and autopsy.

tized to some constituent of plasma obtained from one or more of the donor dogs.

The other 2 dogs in this hypoproteinemic group (Nos. 38-25 and 39-27⁷), although the experimental procedure was identical, did not develop arterial lesions and therefore are not included in this paper. The only known difference for these dogs was that the dosage of uranium nitrate was less: in No. 38-25 it was 2.5 mg. per Kg. and in No. 39-27, 2.0 mg. per Kg. Thus all dogs in all groups (see following for Group B) which in addition to some alteration in their plasma proteins received 3.0 mg. or more of

1). Occasionally, however, the outlines were irregular and in some instances extension into the media could be seen with the unaided eye. Engorged tiny vessels, hemorrhage and a scant amount of fibrin could sometimes be detected in the adventitia. Thrombosis was the exception rather than the rule. No gross evidences of calcification were encountered in these regions. In the aorta the lesions were limited to the ascending portion, the first half of the arch and the abdominal aorta. The thoracic portion of the descending aorta was not involved. The lesions were usually most marked in the first portion of the ascending aorta. Occasionally they completely surrounded the orifice of one of the coronary arteries, but in no instance was there any narrowing of the orifice. The sinuses of Valsalva and of the pulmonary valve, when involved, appeared bulged outward and irregularly wrinkled or showed only one or two small grayish brown, raised areas. In the pulmonary aorta, as in the ascending aorta, the lesions were more pronounced in the first portion and did not extend beyond the bifurcation. The lesions in the lungs (Fig. 12) were found only microscopically.

Under the microscope it was difficult to determine just how the process began. There appeared to be an injury to the subendothelial connective tissue with pyknosis of the nuclei and swelling of the cells and fibers in this tissue (Fig. 2). No doubt there was also some injury to the endothelial lining with increase of its permeability, for edema, fibrin, and hemorrhage soon made their appearance. Subendothelial deposits of fibrinoid material were sometimes seen (Fig. 3). The fully developed process, which was the one usually encountered, presented massive necrosis with destruction of the intima, often extending deeply into the media, and there was usually some inflammatory reaction in the adventitia. The predominant recognizable leukocyte in this reaction is the polymorphonuclear neutrophil, but the majority of the cells are most accurately described as "cells with distorted nuclei." Fully developed eosinophils were not seen and only occasional eosinophilic myelocytes were encountered. In the more advanced lesions, large mononuclear cells were numerous and there was some enlargement, basophilic staining and proliferation of the fixed tissue cells (fibroblasts and endothelial cells), indicating attempts at repair. The destructive changes, including massive

Clinical evidence pointing to the arterial lesions was encountered in only one dog, and in this case the symptoms and signs were referable to thrombi which formed over the arterial lesions. During the 48 hours preceding death, dog No. 38-24 developed progressively gangrene of the anterior third of the tongue and paralysis of both hind legs and the right foreleg. The affected extremities were cooler than normal and no arterial pulsation was palpable in them. None of the other 7 dogs showed any clinical manifestations referable to the arterial lesions. The chief cause of death in all instances was presumably "uremia." The figures for the terminal nonprotein nitrogen and the extensive necrosis of the proximal convoluted tubules seen in histological sections of the kidneys substantiate this clinical impression.

GROSS AND MICROSCOPICAL DESCRIPTION

As indicated in Table II, the lesions were found principally in the aorta, sinuses of Valsalva, pulmonary artery and in the endocardium of the left auricle and were strikingly absent from the parenchyma of organs other than the lungs and heart. Involvement of the coronary arteries was confined to the larger branches. The innominate, carotid, subclavian, internal mammary, femoral and mesenteric arteries were examined in all but one of the dogs. Gross lesions were found in only one dog (No. 38-24) but as indicated in Table II, histological lesions were found in the mesenteric artery in two other dogs and in the carotid artery in one of these. Thus both elastic and muscular arteries were subject to the injury, but the lesions were more prevalent and more prominent in the elastic arteries. (The structure of the left auricle warrants its inclusion in the category of elastic arteries.) No lesions were found in veins or lymphatics.

The lesions in the ascending aorta, sinuses of Valsalva and pulmonary artery were similar in gross and histological appearance. When the vessels were opened the lesions appeared on the intimal surface as slightly raised, wrinkled, inelastic, grayish brown patches which contrasted sharply with the smooth intima about them. They varied in size from 1 to 2 mm. to 2 to 3 cm. in maximum diameter and were sometimes confluent (Fig. B). Frequently they were round or oval in outline and sometimes they were sharply defined and apparently limited to the intima (Fig.

were similar to those described (Figs. 4-6). Possibly because the tissues in this region were less compact than those in the aorta, the deposition of fibrin and fibrinoid material and the infiltration of leukocytes was a little more conspicuous. Similar, though less marked, exudate sometimes extended out into the interstitial tissue of the subjacent myocardium. While the cardiac fibers themselves were not usually involved, in two of the dogs a rather extensive necrotizing myocarditis involving the septum, papillary muscles and wall of the left ventricle, was encountered. Also in two instances the mitral valve showed acute necrotizing valvulitis. Collections of large mononuclear cells, sometimes mixed with polymorphonuclear leukocytes, occurred in the perivascular connective tissue of the myocardium, but none of these collections had the characteristics of an Aschoff body.

In the abdominal aorta (Fig. A) and in some of the other sites tabulated in dog No. 38-24 (innominate, subclavian, femoral, and internal mammary arteries), the gross appearance was somewhat different. Even before the vessels were opened, marked irregularity was obvious. The crumpled, inelastic, irregularly dilated and sometimes beaded appearance of the vessels contrasted sharply with the normal tubular or uniformly collapsed appearance. On opening the vessels, the crumpled, irregularly dilated character was even more manifest; and as the vessels were opened the scissors occasionally gritted as though cutting through very thin egg shells. The endothelium was intact save at the sites of thrombosis (innominate and both femoral arteries); and when viewed from the intimal surface, the vessel wall was less translucent than normal.

On histological examination of these vessels, the outstanding features were necrosis and deposition of calcium. At once was noted the loss of the normal wavy pattern of the internal elastic membrane. The normal folds of the intimal surface of these arteries, as seen in cross section, were obliterated. This change may involve only a portion or the entire circumference of the vessel. The nuclei in the media were fading or gone and the tissue in this region either stained more intensely with eosin or was becoming hyalinized and impregnated with calcium salts. Figure 11 shows an intense deposit of calcium in the necrotic media of one of the femoral arteries. The lumen at this site was completely

karyorrhexis and karyolysis with the formation of pools of nucleic acid, so far overbalanced the proliferative changes that the finding of healed lesions in the pulmonary artery of dog No. 39-28 (Fig. 7) was a distinct surprise. The gross and especially the histologic changes suggested an aneurysm as a more likely possibility but no definite aneurysms were encountered. Other surprising features were that the endothelial lining remained intact as long as it did and that thrombi were not more frequent.

Elastic tissue stains showed that in the earliest lesions the elastic framework was intact, but instead of the usual wavy contour the fibers were straight. Very soon, however, the internal elastic membrane became frayed and fragmented and as the lesion progressed, bits of the fragmented elastic fibers broke off into the necrotic debris and apparently underwent lysis. In the most extensively involved area, the elastic framework of practically the entire thickness of the media of the aorta had been destroyed. Figure 10 illustrates the margin of this area. Fat stains were negative. Von Kossa stains have revealed the presence of tiny black calcium granules along the course of some of the disintegrating elastic fibrils, but these were not so numerous as the hematoxylin and eosin preparations suggested. Numerous Gram and methylene blue stains of the most promising lesions have failed to reveal any organisms. The connective tissue stains, both van Gieson's and Mallory's, showed about equal involvement of smooth muscle and collagenous tissue.

In the endocardium of the left auricle the lesions appeared as tiny, punctate or streaky, slightly raised, yellowish opacities and were usually grouped in an area 1 to 2 cm. in diameter above the posterior leaflet of the mitral valve (Fig. D). In one case similar lesions were present in the left auricular appendage. In all three instances in which lesions were found in this chamber, the endocardium over them appeared to be intact and there was no adherent thrombus. Subendocardial hemorrhages were not infrequent in the left auricle but were usually more numerous in the left ventricle and have been found in the mitral and aortic valves (Fig. B). Hemorrhages were also encountered in the epicardium, especially along the right atrio-ventricular groove and about the base of the aorta.

Histologically the lesions in the endocardium of the left auricle

only a scant infiltration of lymphocytes and large mononuclear cells persisting. The endothelial surface over these "scars" was everywhere intact. There were no capillaries and no old phagocytosed blood pigment in any of the "scars." The media and adventitia appeared entirely normal. Several of the arteries in the lungs showed similar fibrous intimal thickenings (Fig. 8), and there were a few areas of fibrosis about the vasa vasorum and in the interstices of the elastic framework of the aorta. The internal elastic membrane in the aorta in all the sections examined was normal, and there were no fibrous intimal thickenings in this vessel. The endocardium of the left auricle and all other blood vessels in the routine sections showed no change. The question whether the arterial lesions of dog No. 39-28 were due to the healing of a previous necrotizing arteritis or were the result of intimal reaction to the parasitic infection, or a combination of the two, must be left open. The idea that these acute lesions can heal is not incompatible with the findings in some cases of periarteritis nodosa.¹⁶

DISCUSSION

The questions of most interest concern the pathogenesis of the lesions and their possible bearing on lesions encountered in human pathology. In regard to the latter point, about all that can be said at this time is that some of the lesions bear some similarity to those of periarteritis nodosa and rheumatic arteritis, but no claim of identity is made. The discussion of the former point is divided into three parts:

1. *Exclusion of certain possibilities of error.* Are the lesions related to the experimental procedure or are they merely coincidental? To rule out the possibility of an epidemic or epizootic of some sort (such as that recently described by Loewe and Lenke¹⁷ in rabbits, with cardiac lesions resembling those of human rheumatic fever), 20 dogs, derived from the same sources and kept in the same quarters at the same time as the experimental animals, were examined. Not one of these had any lesions in its arteries. Furthermore, despite the fact that these experiments have been in progress for over a year, both the gross and histologic appearance of the lesions has in all instances corresponded to the time interval between the injection of uranium nitrate

occluded by a thrombus. In and about some of these necrotic areas were appreciable infiltrations of polymorphonuclear leukocytes, apparently coming both from the lumen and from the vasa vasorum, but in most of the sections examined, necrosis and calcification overshadowed leukocytic infiltration.

In only one instance, dog No. 39-28, was there an opportunity to examine other than the acute lesions described above, and in this case there was a complication which may invalidate the findings. Despite the fact that this dog was subjected to the same experimental procedures as the other dogs in Group A; *i.e.*, the same basal diet, repeated injections of plasma obtained from the same group of donors, and the same dosage (5.0 mg. per Kg.) of the same standard solution of uranium nitrate, at no time was there any clinical evidence of illness. The usual proteinuria followed the injection of the heavy metal and by the eighth day the nonprotein nitrogen had risen to 92 mg. per 100 cc., yet the dog continued to eat all of its diet each day, body weight was maintained, and the general clinical condition was good throughout. The dog was kept on the basal diet for 9 weeks, by which time the plasma protein concentration had dropped to 6.6 per cent and the nonprotein nitrogen had fallen to 36 mg. per 100 cc. It was then returned to the regular kennel diet until it was sacrificed with ether 11 months after the uranium nitrate injury and almost exactly 1 year after the first injection of donor's plasma. At autopsy, the remarkable lesions depicted in Figure C were found in the pulmonary aorta. There were literally hundreds of tiny grayish white tags, everywhere covered by smooth white endothelium, which gave the intimal surface a shaggy appearance. It is noteworthy that no thrombi formed over this very irregular surface.

The complication in this dog consisted of the presence of four living adult female "heart worms" (*Dirofilaria immitis*) coiled up in the lumen of the pulmonary artery. Other cases of this infection seen in this laboratory and cases reported in the literature¹⁵ have not shown the anatomical changes illustrated in Figure C. Hence it is reasonable to assume that there had been previously an acute arteritis similar to that which appeared in the other 4 dogs in this group and that it had "healed" with the residual fibrous intimal "scars" shown in Figures 7 and 9. These "scars" were confined to the intima and consisted of fibrous tissue with

plasma, it is conceivable that it might play a rôle in the production of the lesions. In 4 of the dogs in the hyperproteinemic group (group A), sodium citrate was used for this purpose. In the other member of this group, dog No. 40-50, heparin was used as the anticoagulant. Identical arterial lesions appeared in this dog. This, plus the fact that lesions developed in 2 hypoproteinemic dogs (group C) in which the amount of anticoagulant injected along with the *washed* corpuscles was negligible, seems to exclude this factor from any pathogenic rôle.

3. *Conjectures on pathogenesis.* No plausible theory of pathogenesis has as yet been developed and no direct evidence of any kind has been obtained. The feature which is most impressive as having some pathogenic significance is the histologic appearance of the lesions. Their similarity to those encountered in anaphylactic and allergic states is striking, but with one notable difference. In these altered states, the acute necrotizing changes are found predominantly in the smaller blood vessels and capillaries and not in the larger arteries and endocardium, as in the experiments reported in this paper. Furthermore, abnormalities correlated with repeated daily injections over a period of 3 to 4 weeks suggest hypersensitivity or altered sensitivity as a mechanism of their production. The possibility that the lesions are due to incompatibilities (such as those described by Melnick, Burack and Cowgill¹⁹ in dogs receiving repeated infusions of red blood cells, or those described by Rous and Robertson²⁰ in rabbits receiving repeated small transfusions) developing from some constituent of the blood of one or more of the donor dogs has to be considered. Records were kept of all bleedings and injections and these were checked with this possibility in mind, but no incriminating evidence was obtained. Although 2 or more of the 10 donor dogs were common to 6 of the experimental animals with arterial lesions, there is no single donor common to all the experiments. No direct observations on cross-matching at different stages in the experimental procedure have been made. Certainly the negative evidence just cited does not exclude the possibility of incompatibilities. But even if positive evidence on this point were obtained, the rôle of uranium nitrate in the production of the lesions would still remain unexplained.

Uranium nitrate could act in one or more of several ways. It

and death: 8 to 17 days for 7 of the dogs and 11 months for dog No. 39-28.

Are the lesions due to something accidentally introduced during the experimental procedure, such as a bacterium, virus, or protozoan? This question, which is especially pertinent since sterile technic was not employed in making the plasma injections or withdrawals, cannot be answered finally; but all available evidence is against this possibility. (a) In dogs inoculated with known bacteria, viruses, or protozoa, such arterial lesions do not develop. (b) Blood cultures of the experimental dogs have been negative. (c) Sections of the lesions stained with bacterial stains have failed to reveal any organisms. (d) Intravenous plasma injected alone, plasmapheresis alone, or subcutaneous injections of uranium nitrate alone do not produce the lesions, and the opportunity of introducing organisms or virus along with these injections is just as great as it is in the experiments reported. These facts seem to exclude every possibility except the accidental introduction of some unknown organism or virus having a peculiar affinity for arteries.

2. *Negative evidence bearing on pathogenesis.* Since all the dogs with arterial lesions were maintained on the standard basal diet throughout the experimental procedure, it is conceivable, although unlikely, that some constituent of the diet enters into the reaction which produces the arterial lesions. (Viosterol lesions in rabbits¹⁸ pointed the finger of suspicion at the cod liver oil which constituted 6.5 per cent of the diet by weight.) To check on this point, 6 dogs were placed on the standard basal diet for 4 weeks and then given 5.0 mg. of uranium nitrate per Kg. Only 1 of the 6 dogs developed arterial lesions and, as previously explained, this dog (No. 39-43) had received 2 intravenous injections of donor's plasma 3½ weeks previously; hence was not a strict control. This dog has been included in the experimental group (Tables I and II), leaving the 5 unequivocal control animals entirely negative. This evidence, of course, does not mean that the diet or some constituent thereof is not a necessary part of the reaction which produces the arterial lesions. This point is being checked at the present time.

Since the anticoagulant used in obtaining donor's plasma is injected into the vein of the experimental animal along with the

lesions in the pulmonary artery when it was sacrificed 11 months later.

2. Control dogs, maintained on the same diet and receiving the same dosage of uranium nitrate (5.0 mg. per Kg.) but not subjected to plasmapheresis or plasma injections, failed to show any arterial lesions.

3. The lesions affected principally the elastic arteries, the commonest sites being the aorta, sinuses of Valsalva, pulmonary arteries and the endocardium of the left auricle. These lesions are strikingly absent from the parenchyma of organs other than the heart and lungs.

4. Essentially, the lesion is an acute necrotizing arteritis usually most marked in the intima but not infrequently involving all three coats. Polymorphonuclear neutrophils predominate in the cellular response. Calcium deposition in the necrotic areas is frequent. Sometimes thromboses occur. Some of the lesions have a semblance to those of periarteritis nodosa and rheumatic arteritis.

5. Bacteriological studies of the blood and bacterial stains of the lesions have failed to reveal any organisms.

6. The possibility of a spontaneous disease, epizootic in nature, seems to be excluded by the control data.

7. The nature of the process is briefly discussed.

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could act as a simple chemical compound to form a "conjugated protein"²¹ with some constituent of the blood of one or more of the donor dogs; and this "conjugated protein" could act as sensitizing antigen. Or, uranium nitrate could precipitate the "reaction" previously prepared by some constituent of the blood of one or more of the donor dogs. Or, the essential action of uranium nitrate could be the production of renal injury, and the essential mechanism in the production of the arterial lesions could be some constituent in the blood of one or more of the donor dogs (or product thereof) acting in the disturbed metabolism consequent to the renal injury. It is of interest in this connection that a considerable percentage of human cases of generalized necrotizing arteritis are associated with diffuse glomerulonephritis.⁵

SUMMARY OF DISCUSSION

The arterial lesions presented in this paper in all probability are not due to accidental or coincidental infection, but seem definitely to be related to the experimental procedure. Diet and anticoagulant can probably be excluded from any pathogenic rôle. The factors common to all of the experiments in which arterial lesions developed are: (1) some alteration in the plasma proteins produced by the intravenous injection of some constituent of apparently "homologous" blood, and (2) uranium nitrate injury (3.0 mg. or more per Kg. administered subcutaneously 3 to 4 weeks after starting the alteration). As a tentative hypothesis it is suggested that the lesions may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs precipitated by uranium nitrate or made manifest in the disturbed metabolism consequent to the uranium nitrate injury.

SUMMARY

1. During experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level it was observed that 8 of 10 dogs, subjected to alterations in their plasma proteins by plasmapheresis or plasma injections and then given uranium nitrate subcutaneously, showed necrotizing arteritis when they died 8 to 17 days later. One of the 8 dogs survived the early phase of the experiment and showed healed

DESCRIPTION OF PLATES

PLATE 69

Necrotizing arterial lesions in dogs.

FIG. A. Dog No. 39-45. Necrotizing aortitis of abdominal aorta.

FIG. B. Dog No. 39-40. Acute necrotizing aortitis of ascending aorta. There is hemorrhage in the septal endocardium extending into the base of the anterior aortic cusp.

FIG. C. Dog No. 39-28. Healed necrotizing arteritis of the pulmonary aorta.

FIG. D. Dog No. 39-40. Acute necrotizing left auriculitis.

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PLATE 70

Necrotizing arterial lesions in dogs. All sections were stained with hematoxylin and eosin.

- FIG. 1. Dog No. 39-40. (See Figure B for site of section.) Acute necrotizing aortitis largely confined to the intima and the inner layers of the media. Note the intact endothelium and sharp border of lesion. $\times 45$.
- FIG. 2. Dog No. 39-40. (From area indicated in Figure 1.) Necrosis and swelling of subendothelial collagenous tissue. There is also some edema. $\times 610$.
- FIG. 3. Dog No. 39-40. (From area indicated in Figure 1.) Deposition of fibrinoid material beneath intact endothelium. Massive necrosis and infiltration of leukocytes, both polymorphonuclear and mononuclear, and cells with distorted nuclei. $\times 150$.
- FIG. 4. Dog No. 39-40. (See Figure D for site of section.) Acute necrotizing auriculitis. Massive leukocytic infiltration and fibrin deposition beneath the intact endothelium; also some polymorphonuclear infiltration of the interstitial tissue of the myocardium. $\times 45$.
- FIG. 5. Dog No. 39-40. (From area indicated in Figure 6.) High power view of the exudate. Cells with distorted nuclei predominate, but polymorphonuclear leukocytes and large mononuclear cells can be recognized. Note the mitotic figure in one of the latter cells near the right lower corner. $\times 610$.
- FIG. 6. Dog No. 39-40. (From area indicated in Figure 4.) Medium power showing intact endothelium, fibrin deposition, leukocytic infiltration and necrosis. $\times 110$.



A

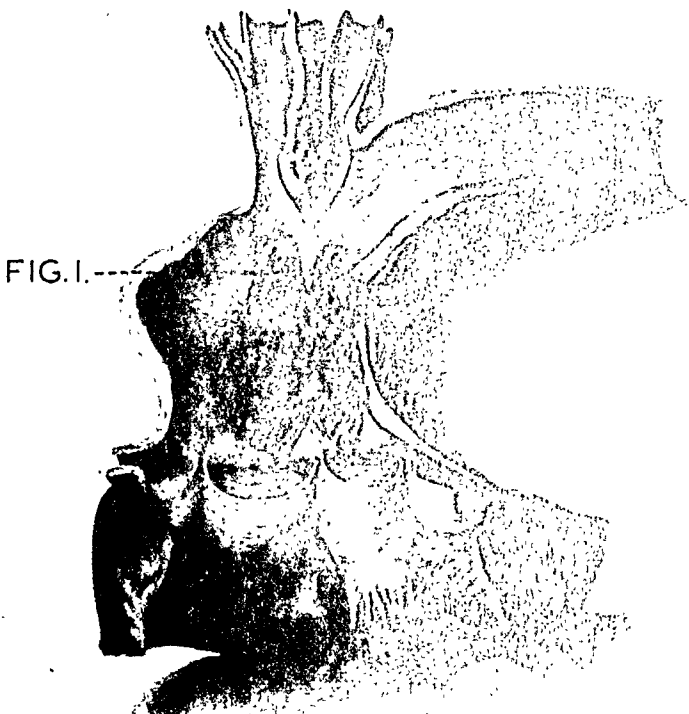


FIG. 1.

B



FIG. 7.

C



FIG. 4.

D

PLATE 71

Necrotizing arterial lesions in dogs. All sections, except that used for Figure 10, were stained with hematoxylin and eosin.

FIG. 7. Dog No. 39-28. (See Figure C for site of section.) Fibrous intimal plaques covered with endothelium. These are presumably healed lesions (see text). $\times 40$.

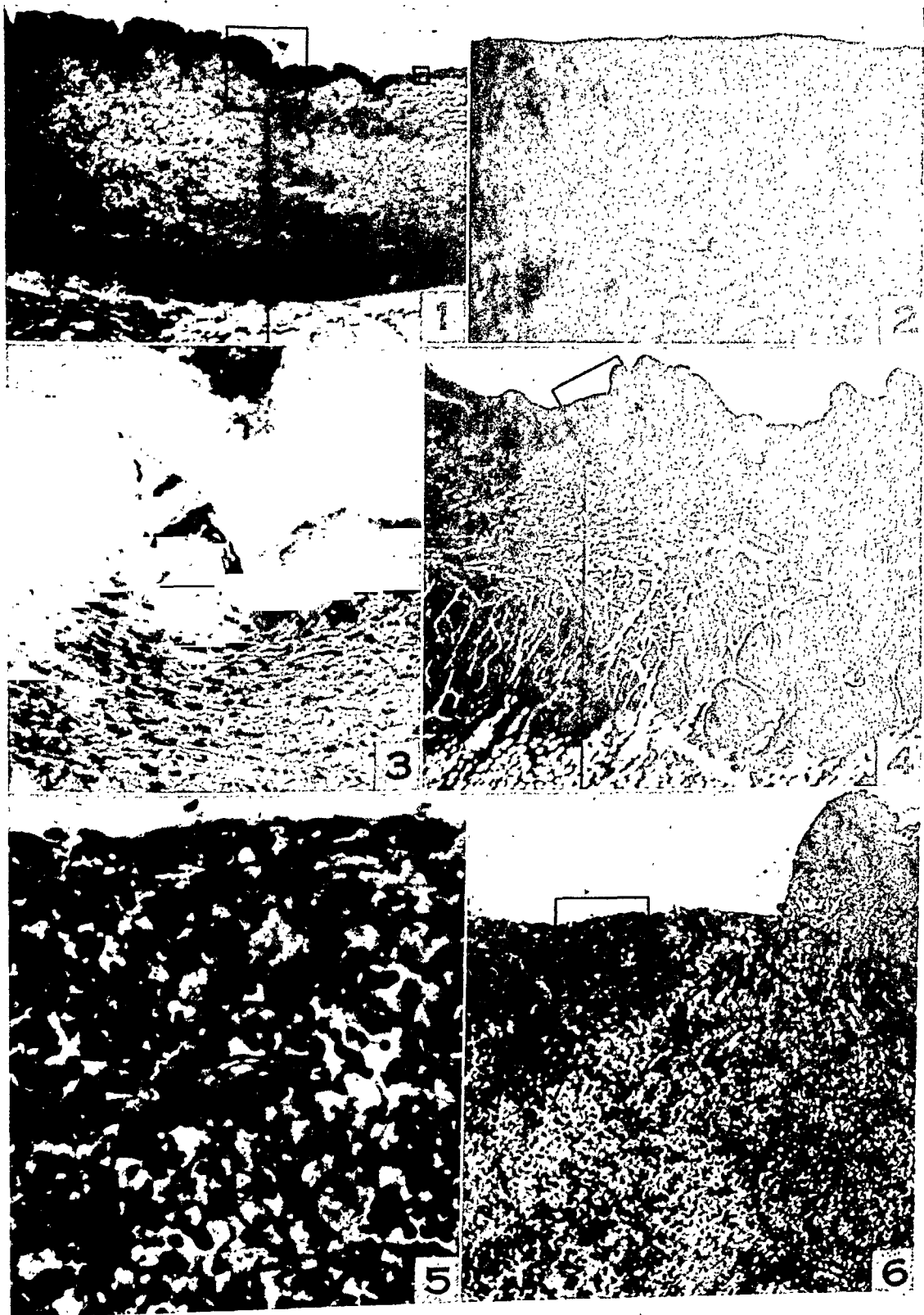
FIG. 8. Dog No. 39-28. Similar fibrous intimal plaque in artery in lung. $\times 110$.

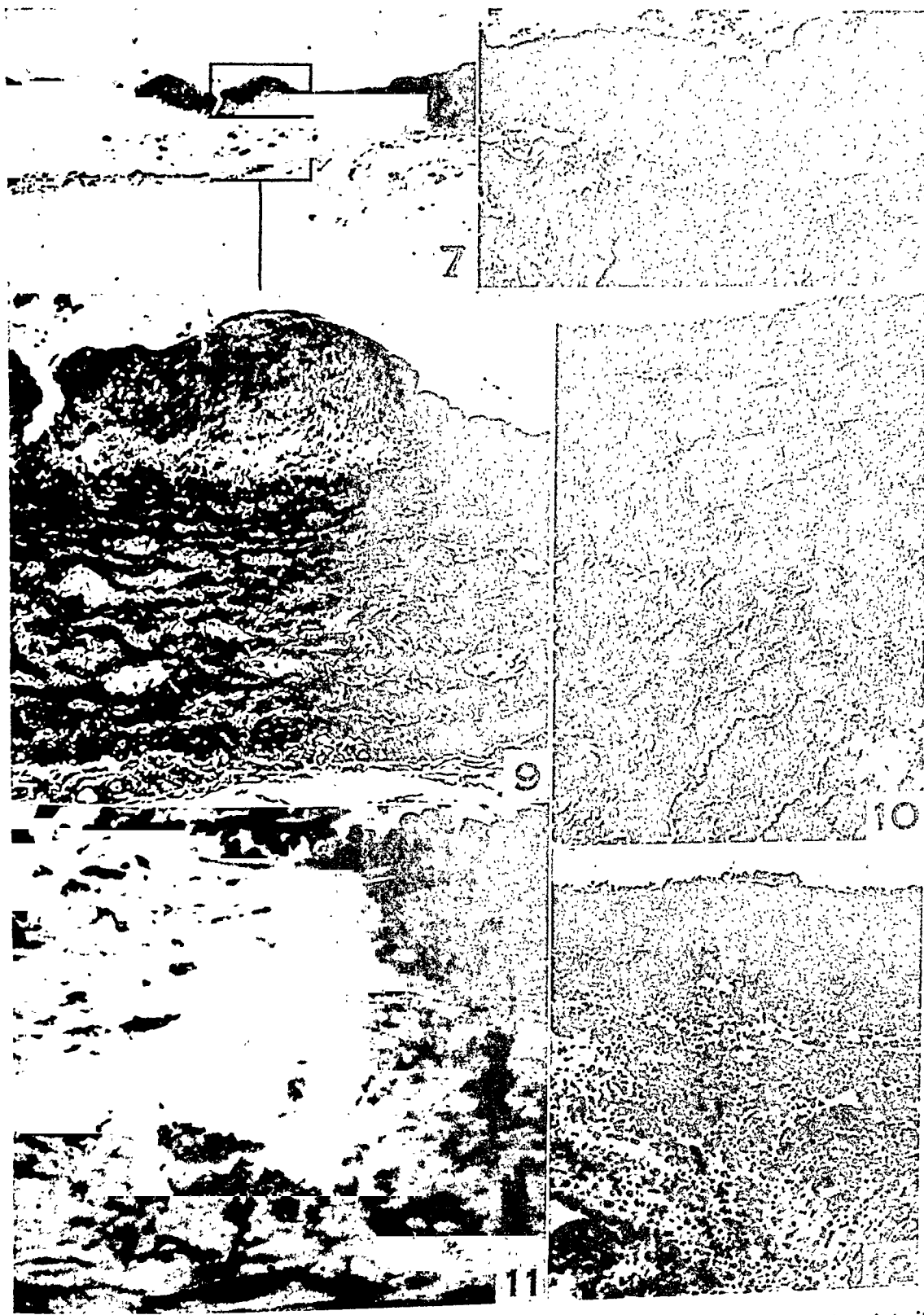
FIG. 9. Dog No. 39-28. (From area indicated in Figure 7.) $\times 150$.

FIG. 10. Dog No. 39-40. Acute necrotizing aortitis showing disruption of elastic framework and leukocytic exudate in deeper layers of media. Verhoeff's elastic tissue stain. $\times 100$.

FIG. 11. Dog No. 38-24. Necrosis, hyalinization and calcium deposition in the media of the femoral artery. The lumen of this vessel was occluded by a thrombus. $\times 610$.

FIG. 12. Dog No. 39-34. Acute necrotizing arteritis and periarteritis in an artery in the lung. Note loss of elasticity (as indicated by straight contour of vessel), massive leukocytic infiltration and necrosis. $\times 110$.





The following controls were employed: 15 rats were given a single intracardiac injection of the same bacterial suspension and were killed at intervals varying from 6 hours to 11 days; 5 rats were given 2 to 4 intracardiac injections of heat-killed organisms; and 5 others were similarly injected with sterile broth. All control animals were killed with ether 4 to 8 days after the first injection.

A group of 14 mice weighing approximately 20 gm. received 16 injections, each consisting of 0.1 cc. of a similarly concentrated culture, by the tail vein, during a period of 3 months. Only 3 of the mice died of intercurrent infection prior to the conclusion of the experiment.

The heart's blood of all rats and mice was cultured in broth after autopsy and paraffin sections of heart, brain, kidneys, and lungs were prepared following fixation in formaldehyde solution. The sections were stained with hematoxylin and eosin. Bacteria were demonstrated by the Gram stain, collagen by Masson's stain, and reticulum by the method of Wilder.

RESULTS

The early reaction (24 to 48 hours) in rats consisted of miliary abscesses in the myocardium, kidneys and brain. The myocardial abscesses in a few animals were associated with diffuse leukocytic infiltration. A diffuse leukocytic infiltration was also present in the epicardium of many rats, particularly at the auriculoventricular sulcus.

Following this initial response there was a rapid replacement of leukocytes by monocytes in the involved organs. This was particularly pronounced in rats which received only one intracardiac injection. Animals given multiple injections exhibited relatively few leukocytes in the myocardium 6 days after the initial injection, and in several instances as late as 17 days after the first intracardiac injection. It was interesting to note that a number of rats given multiple injections failed to exhibit a leukocytic response in the heart or elsewhere, although no more than 24 to 48 hours had elapsed since the last intracardiac injection.

More than one third of the rats given four or more intracardiac injections exhibited myocardial lesions which were not found in control animals given only one injection. The lesions, usually in

THE CYTOLOGIC RESPONSE OF RATS AND MICE TO A STRAIN OF GREENING STREPTOCOCCI*

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Numerous experiments, designed to test the etiologic relationship of strains of greening streptococci to rheumatic heart disease, have centered attention on focal collections of mononucleated cells in the myocardium found particularly in rabbits. Some investigators¹⁻⁴ maintained that these cellular aggregates resemble Aschoff bodies so closely as to be identical with them, whereas others⁵⁻⁸ thought that these inflammatory lesions differ from those of rheumatic fever and that any resemblance to Aschoff bodies was without diagnostic significance.

The present study of myocardial lesions with associated changes in other organs in rats and mice, following infections with greening streptococci, is reported because it seems to throw additional light upon the significance of such findings in other animals.

MATERIALS AND METHOD

A strain of greening streptococci, recently isolated from a case of subacute bacterial endocarditis, was grown on neopeptone beef broth of P_H 7.6 for 48 hours and used without previous animal passage. Intracardiac injections of this organism were given three to five times a week to 25 rats weighing from 125 to 150 gm. The material injected consisted of the centrifugalized bacterial sediment from 0.6 cc. of a 24-hour culture resuspended in 0.3 cc. of the supernatant broth. The number of such injections varied from 4 to 19 per rat and the life of the animals varied from 4 to 58 days. The intracardiac injections were done under light ether anesthesia with a tuberculin syringe and a 26 gauge needle. The site of puncture was prepared by wetting the hair and skin with alcohol. Some of the rats given multiple intracardiac injections died of cardiac tamponade during the course of the injections; others died spontaneously of the induced disease; whereas the remainder were killed with ether.

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contained hemosiderin and were situated in areas where there was an apparent loss of brain substance. Some extracellular hemosiderin was also present.

The kidneys of rats given single infective doses showed lesions qualitatively identical with those of animals subjected to multiple injections. The latter exhibited interstitial inflammatory foci in addition to a classical focal embolic glomerulonephritis. The leukocytes present 24 hours after injection were quickly replaced by monocytes; so that 5 or more days after the initial injection, most animals exhibited only healing or healed glomerular lesions (Fig. 4) in spite of numerous superimposed, relatively recent intracardiac injections.

Although bronchitis, peribronchitis, and pneumonia were not uncommon findings in both infected and control rats, 10 of 25 which received multiple intracardiac injections of living organisms showed lesions not seen in other animals. These lesions were composed of mononucleated cells and Langhans' giant cells grouped to form miliary nodules which contained no bacteria.

Rats injected with sterile broth or with heat-killed cocci showed no lesions other than occasional myocardial scars.

Three fourths of the mice given repeated intravenous injections with suspensions of greening streptococci exhibited focal monocytic infiltrations of the myocardium but no endocardial vegetations or foci resembling Aschoff bodies. Occasional myocardial scars and, more frequently, focal epicardial infiltrations by monocytes and lymphocytes were additional findings. Less than one half of these mice showed brain lesions. These consisted of small areas of degeneration or necrosis infiltrated by monocytes and occasional leukocytes. Occasional vessels exhibited "collars" of similar cells. Although focal, embolic, glomerular lesions were not found in the injected mice, nearly all exhibited small, interstitial, monocytic foci.

DISCUSSION

The salient result of multiple intracardiac injections of greening streptococci was the monocytic reaction in the heart, brain, kidneys and lung, which was preceded by a transient leukocytic response. Focal myocardial lesions resembling Aschoff bodies were observed in rats but not in mice. These lesions differed from

interstitial and occasionally in perivascular regions in the ventricular myocardium, consisted of focal collections of large, epithelioid, nonphagocytic cells with abundant, somewhat basophilic cytoplasm, poorly defined cell boundaries and large oval nuclei. A heavy nuclear membrane and a prominent, frequently stellate nucleolus contributed to the close resemblance to the cell described as characteristic of the Aschoff body (Fig. 1). However, remnants of degenerated or necrotic collagen in these foci and the "chicken-wire" network of reticulum stressed by Louis Gross and Ehrlich⁹ as important criteria of the Aschoff body, were not demonstrable in sections stained specifically for these substances.

Valvulitis and mural endocarditis, often with massive vegetations, were encountered in 10 (40 per cent) rats subjected to multiple intracardiac injections (Fig. 2). The vegetations consisted of fibrin, leukocytes, debris, and numerous bacterial masses. Varying degrees of organization were present near the endocardial attachment in some vegetations. In 2 rats an acute purulent endoarteritis was seen a short distance above the valve.

Myocardial scars were the most constant finding in nearly all rats 4 or more days following single or multiple intracardiac injections with living or heat-killed streptococci, or with sterile broth. The character of the scar varied, of course, with its age. Animals that survived 2 weeks or longer exhibited well-contracted, collagenous scars containing some hemosiderin, practically no inflammatory cells, and few adult connective tissue nuclei.

The evanescence of the leukocytic response to this strain of greening streptococci was also demonstrable in the brain. Whereas masses of leukocytes surrounding bacterial clumps were characteristic of brain lesions 24 hours old, 4 days later the leukocytes were nearly all replaced by monocytes and the bacteria had completely disappeared (Fig. 3). Rats that lived 9 or more days after the initial injection and were subjected to numerous subsequent injections exhibited no lesions which could be identified by the type of cell present as of more recent origin; nor were the observed lesions per section more numerous in animals which received 15 intracardiac injections than in those which had been injected only once or twice. At this time the brain lesions consisted of small clumps of large monocytic phagocytes which

SUMMARY

The cytologic response in the rat to intracardiac injections of living cultures of greening streptococci was predominantly monocytic, preceded, however, by a transient leukocytic reaction. In the myocardium of many animals, structures resembling Aschoff bodies were seen. This resemblance was not significant because of the absence of degenerated collagen and the "chicken-wire" network of reticulum, considered important criteria of the Aschoff body.

A similar response, but much milder in degree, was observed in mice subjected to repeated intravenous injections of the same organism. Lesions simulating the Aschoff body were not found in these animals.

A study of the lesions produced by a strain of greening streptococci in rats and mice, correlated with reports of similar lesions in other animals, leads to the belief that the monocytic response in the rat, rabbit, and probably other animals following the injection of these microorganisms is merely the reaction of an immune or partially immune host to a pathogen of relatively low virulence and that the formation of giant cells, as well as structures superficially resembling Aschoff bodies, is a minor modification of this general reaction.

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true Aschoff bodies in several important respects and appeared to be merely a slight modification of the monocytic response provoked by the greening streptococci in other organs. The peculiar miliary nodules of monocytes and Langhans' giant cells seen in the lung represented still another modification of the same monocytic response.

A mobilization of large monocytes in association with Langhans' giant cells was described by Nye and Parker¹⁰ following repeated intravenous injections of nonhemolytic streptococci in rabbits. The experimental results obtained by Louis Gross, Loewe and Eliasoph⁸ led these workers to conclude that "the rabbit and possibly other animals, are peculiarly prone to the formation of multinucleated cells in collections somewhat resembling Aschoff bodies following the injection of a variety of toxic substances."

This statement may be paraphrased to the effect that the monocytic response seen in the rat, rabbit, and probably other animals following the injection of greening streptococci is characteristic of the reaction of an immune or partially immune host to a pathogen of relatively low virulence; and that the formation of giant cells as well as of structures resembling Aschoff bodies is merely a minor modification of this general reaction.

Indications of such immunity are seen in the failure of most rats to respond with new abscesses in the various organs following repeated intracardiac injections of living streptococci.

No particular significance or importance is attached to the presence of endocardial vegetations in 28 per cent of the rats. Various workers have produced endocardial vegetations in dogs and rabbits by a combination of cardiac trauma with intravenous injections of greening streptococci and in this respect the production of vegetations in these rats was similar.

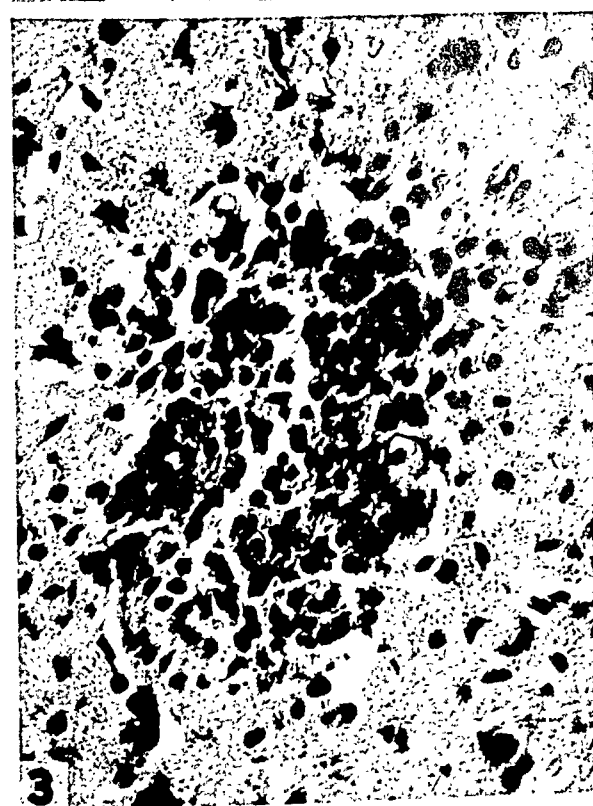
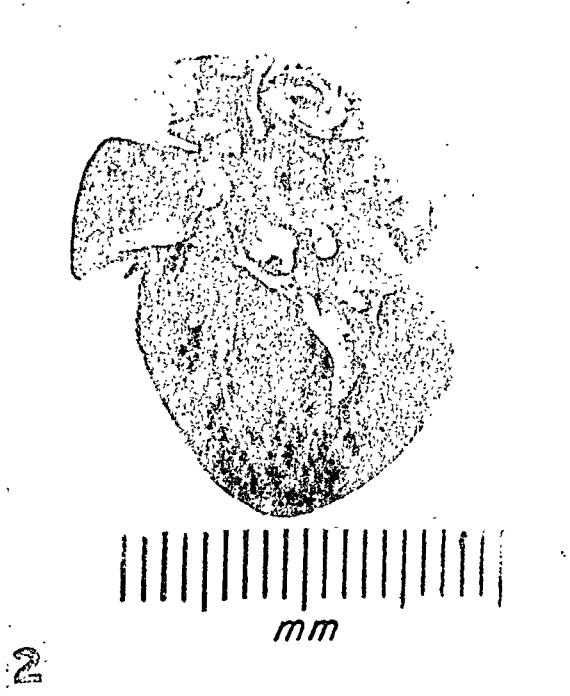
The cytologic response to greening streptococci in mice was qualitatively similar to that seen in rats, except that lesions simulating Aschoff bodies and miliary nodules in the lungs were not observed. Endocardial vegetations were not found in mice, probably because the heart was not traumatized. The cellular reaction in the rat was more severe than in the mouse, probably because the trauma of the intracardiac injections often established foci which favored a more lasting bacteremia than existed with a simple intravenous injection.

DESCRIPTION OF PLATE /

PLATE 72

- FIG. 1. Myocardial lesion resembling an Aschoff body in a rat subjected to 8 intracardiac injections with a strain of greening streptococcus. The animal died 4 days after the last injection, but survived the first injection 18 days. Hematoxylin and eosin stain. $\times 500$.
- FIG. 2. Vegetations, polypoid in character, upon the mitral valve and the left auricular endocardium in a white rat given 17 intracardiac injections with greening streptococci. The animal survived the first injection 38 days and died 21 days following the last injection.
- FIG. 3. Brain lesion consisting entirely of monocytes in a rat given 5 intracardiac injections with greening streptococci. The animal survived the first injection by 9 days and the last by 2 days. Hematoxylin and eosin stain. $\times 330$.
- FIG. 4. Healing, partial glomerular infarction in a rat given 9 intracardiac injections with greening streptococci. This animal survived the first injection by 14 days and the last by only 1 day. Hematoxylin and eosin stain. $\times 380$.

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Gross, Cooper and Phillips

Response to Greening Streptococci

the term "reticulum cell sarcoma." The papers of Oberling so focused attention on the subject that Delbet⁵ in France was led to question "whether there is not nowadays a sort of intoxication which leads one to see more reticulum cell sarcomas than there actually are." Soon afterwards, Roulet^{6,7} wrote his papers on "Retothelsarkom" of the lymph nodes. The frequency with which more and more reticulum cell sarcomas had been described (culminating in the long treatise of Pittaluga⁸ who devoted a good part of his book, which appeared in 1934, to tumors of the reticulo-endothelial system) led Bianchi⁹ to criticize this tendency. His article redelineating the classic concepts of histogenesis of lymphoid tumors is worth reading.

The classification of the Lymphatic Tumor Registry¹⁰ defines the reticulum cell sarcoma as a malignant tumor of reticulocytes (monocytes) and implies that it is closely related to the sarcomatous form of Hodgkin's disease.

For the diagnosis of reticulum cell sarcomas we use the rather restricted criteria given by Oberling² in his original description of "réticulo-sarcome indifférencié." These tumors have the following characteristics: The new growth is formed of syncytial masses of an undivided or slightly fenestrated protoplasm whose limits are ill-defined and connected with zones of more or less differentiated lymphoid tissue. The fenestration, by exaggerating the internuclear spaces, may give a reticular structure. In these undivided masses of protoplasm there are many irregularly distributed oval or indented nuclei, with a well defined nuclear membrane, sparse powdery chromatin granules, and one or two prominent nucleoli. Not infrequently the nuclei give the impression of being empty because of the striking contrast of the darkly stained nuclear membrane and the scanty and lightly stained chromatin. These nuclear characteristics are helpful in differential diagnosis among many other types of malignant cells in which nuclear hyperchromatism is prominent. Some mitoses and occasionally some isolated tumor giant cells also may be found. Mitoses never appear in great numbers. It is not rare to see some lymphocytes scattered throughout the syncytium.

The silver methods bring out variable types of reticulum. These syncytial protoplasmic masses may show a dense network or only scattered, fine, fragmented threads of reticulum.

RETICULUM CELL SARCOMA OF LYMPH NODES*

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Is it justifiable to extend the term "reticulum cell sarcoma" for tumors of lymph nodes as widely as seems to be the tendency in present literature? We believe it is not, since the term cannot be proved to be either proper from the standpoint of general tumor nomenclature or useful from the anatomico-clinical point of view.

It is our hope that, by adopting sharp criteria for the diagnosis of reticulum cell sarcoma of lymph nodes, it will be possible in the future to study with some accuracy the prognosis and radiosensitivity of this type of lymphoid tumor. At present, owing to confusion in nomenclature, the data in medical literature are by no means clearly stated. In view of this growing tendency to use inexactly the term "reticulum cell sarcoma," once sharply defined but now broadened to include almost any lymphoid tumor with proliferation of reticular tissue regardless of degree, we have re-examined a group of 710 lymphoid tumors[†] and have selected for study those fulfilling the more rigid initial criteria.

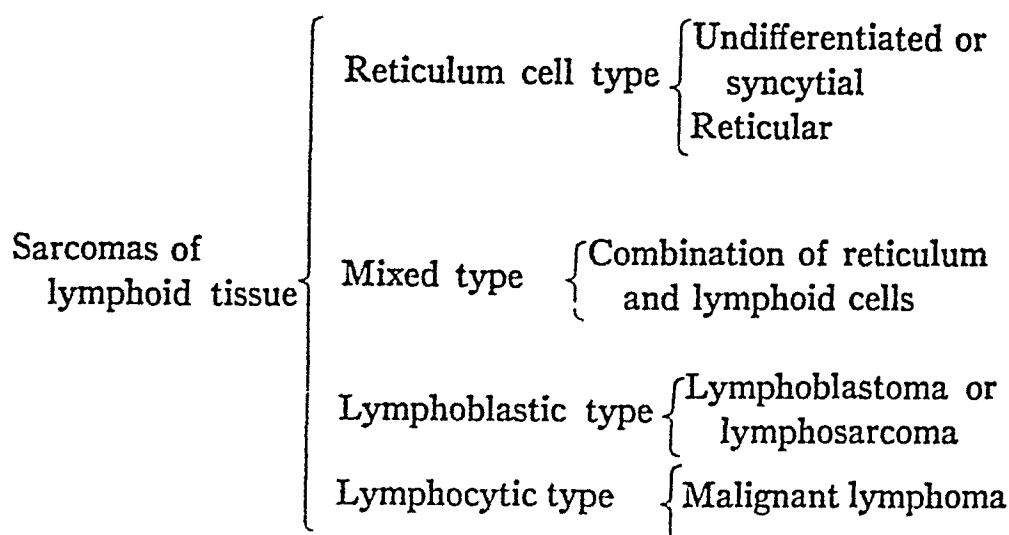
Soon after Aschoff's delineation of the reticulo-endothelial system as a physiological unit, attempts were made to describe special pathological entities of the system. Attention was focused on certain tumors whose constituent cells showed morphologic characteristics reminiscent of the histologic and physiologic properties of the reticulo-endothelial cells.

Goormaghtigh¹ was the first to describe a "malignant proliferation of the reticulo-endothelial tissue of lymph-nodes" made up of cells "different from the round mobile elements of the other benign or malignant lymphoid tumors," and proposed the name "réticulo-endothéliome." Because it implied a tumor of the reticulo-endothelial system as such, this term has met objections. Oberling² and Oberling and Raileanu³ in France used the term "réticulo-sarcome," and Parker and Jackson⁴ in this country popularized

* Received for publication October 17, 1940.

† These tumors were from the files of the Huntington Memorial, Peter Bent Brigham, New England Deaconess and Pondville Hospitals.

ferentiated form of the lymphoid tumors according to this schema:



We have attempted to diagram this relationship in Figure 3, which is synthesized from 5 cases representing each of the types of tumor included in the classification.

The assumption that these tumors are best considered as endotheliomas because they have been seen to arise from the lining cells of the lymph sinuses can be rejected. It is generally accepted that both the lining cells of the sinuses and the reticulum cells of the pulp or germinal centers are embryologically, morphologically and physiologically the same element.¹⁸ If tumors of this sort were derived only from the endothelial cells of lymph sinuses they would not appear in closed lymphoid formations such as the tonsil. One of our specimens (No. 5 in Summary of Cases) comes from a tonsil.

Our concept of the normal lymphoid tissue is a network of undifferentiated cellular reticulum (stem tissue) in whose interstices lie lymphoid cells (lymphoblasts, prolymphocytes, lymphocytes), the offspring of the former. Thus, the complex of *reticulum cells-lymphoid cells* cannot be divorced without destroying the concept of the essential unity of the lymphoid tissue. Any malignant proliferation of lymphoid tissue must then be composed not only of lymphoid cells alone (as is sometimes assumed) but also of reticulum cells. Since the cellular reticulum is the vital constituent, being the stem tissue rather than a simple stroma of the normal lymphoid structure, a tumor of the lymph-

The neoplastic proliferation of reticulum cells presents some typical features that enable one to relate the new growth to the normal reticulum cells. Thus, the masses of undivided or reticulated syncytial cells reproduce the primitive character of the mesenchymal syncytium.¹¹⁻¹³ The nuclei keep their embryonal character, typical also of the reticulum cells.

One of the characters of reticulum cells more frequently quoted, and therefore ascribed to their new growths, is the property of forming argentaffective reticulum fibrils.¹⁴⁻¹⁶ While this is true of these tumors, it is by no means pathognomonic of them.¹⁷ The erroneous assumption that a new growth of lymph nodes showing reticulum proliferation must be classified as a reticulum cell sarcoma is the source of many misleading descriptions. Furthermore, one must not attach too much importance to the production of fibrillar reticulum, paradoxical as this statement may seem. Very undifferentiated reticulum cell sarcomas may show little reticulum within the syncytial masses (Figs. 1 and 2). On the other hand, we are all aware of the striking reticulum proliferation in Hodgkin's disease and in the stromal reaction of the common epidermoid carcinomas, not to speak of many other processes.

In other words, we think that while the presence of reticulum formation affirms the reticular nature of the syncytium, its absence or rarity does not exclude the diagnosis. In the absence of reticulum, special care must be taken to exclude the possibility of the tumor being metastatic from a highly undifferentiated carcinoma.

Other anatomical and physiological properties of the reticulo-endothelial system (phagocytosis, mobilization, lining of lymph sinuses) are not to be considered essential for diagnosis, for otherwise almost any tumor having a mesenchymal origin could be called a reticulum cell sarcoma. The primitive mesenchymal cell may present in its evolution almost any of the characteristics commonly referred to the reticulo-endothelial system. Not only must the reticulum cell sarcoma be differentiated from mesenchymal tumors in general, but from other lymphoid tumors as well. In distinguishing it from tumors of the latter group, we place the dividing line at the stage where appreciable lymphopoiesis can be detected.

Thus we regard the reticulum cell sarcoma as the most undif-

observed and described long ago, albeit at times under various names ("Synzytium-Endotheliom"—Ciaccio;²⁷ "réticulo-endothéliome"—Goormaghtigh;¹ "réticulosarcome"—Oberling;² "Retothelsarkom unreife Form"—Roulet⁶).

APPENDIX

SUMMARY OF CASES

1. Female, 31 years of age. No. 40-1704.
Initial location: neck.
First symptoms developed January 1940.
No follow-up.
Histology: reticulum cell sarcoma (undifferentiated type).
2. Male, 40 years of age. No. 24-1590.
Initial location: neck.
First symptoms developed August 1924.
No follow-up.
Histology: reticulum cell sarcoma (undifferentiated type).
3. Male, 30 years of age. No. 40-1768.
Initial location: neck. Mass in left supraclavicular fossa. Some shotty nodes in axilla and groins.
First symptoms developed July 1923.
Death: October 20, 1923.
Duration: 3 months.
Histology: reticulum cell sarcoma (zones of undifferentiated type).
4. Female, 68 years of age. No. 32-1137.
Initial location: groin.
First symptoms developed January 1932.
Death: January 13, 1935.
Duration: 3 years.
Histology: reticulum cell sarcoma (undifferentiated type).
5. Female, 50 years of age. No. 37-1240.
Initial location: tonsil.
First symptoms developed May 1935.
Patient alive and well March 30, 1940.
Histology: reticulum cell sarcoma showing some differentiation (many lymphoblastic cells).
6. Female, 72 years of age. No. 36-311.
Initial location: neck.
First symptoms developed January 1936.
No follow-up.
Histology: reticulum cell sarcoma, somewhat more reticulated type (periphery well preserved).
7. Male, 17 years of age. No. 38-1644.
Initial location: left axilla. (Widespread to neck, axillary, inguinal and mediastinal nodes.)
First symptoms developed April 1938.

oid cells does not contain reticulum cells only as a supporting element (stroma), but as a part of the tumor itself. These reticulum cells may be either survivors of the original lymph node structure or active participants in the tumor.

The sarcomas of lymphoid tissue can best be classified by the predominant cell type. Malignant proliferation of lymphoid cells of varying degrees of differentiation may occur. These may present different features, although closely related by their histogenesis. This genetic relation to one another should not be permitted to impair the utility of a histologic differentiation, although they cannot be sharply divided. Figure 3 shows their relationships.*

With regard to the structure of the common lymphosarcoma, it is important to remember that in describing it Kundrat¹⁹ first, and Paltauf,²⁰ Sternberg,²¹⁻²³ Banti²⁴ and Aresu and Scalabrino²⁵ afterward, mentioned the presence of both lymphoid cell and reticular cell proliferation. A particularly clear drawing in Ziegler's²⁶ textbook is eloquent enough. We believe that the statement by Goormaghtigh,¹ that the tumors he described were made up of cells different from those which constitute benign and malignant proliferations of lymph nodes, may have led to the erroneous idea that the lymphosarcomas were composed of nothing but lymphoid cells. The rediscovery that reticulum cells are often present in lymphosarcomas has, on the other hand, led to overemphasis of the reticulum cell sarcoma.

Of a series of 710 lymph node tumors examined in this study, 402 were discarded for various reasons. Many were cases of Hodgkin's disease. The rest, 308, were lymphoid tumors suitable for study. Both lymphatic leukemias with lymph node involvement and leukosarcomas were included. Among these we have been able to find but 11 examples of reticulum cell sarcoma, an incidence of 3.6 per cent, much less than is often reported. (See Summary of Cases.) This is due to the restricted use we make of this term.

This conception of the reticulum cell sarcoma is not original with us, nor has the nomenclature been modified by us. The tumor we recognize as a syncytial reticulum cell sarcoma was

* We have omitted Hodgkin's disease which we consider a more complicated process and still controversial.

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Death: February 2, 1939.

Duration: 10 months.

Histology: reticulum cell sarcoma (with zones of undifferentiated type).

8. Male, 44 years of age. No. 36-1967.

Initial location: auricular gland (widespread).

First symptoms developed in 1936.

Death: 1937.

Duration: 1 year.

Histology: reticulum cell sarcoma (somewhat differentiated zones of reticulated type).

9. Female, 54 years of age. No. 24-654.

Initial location: neck.

First symptoms developed April 1924.

No follow-up.

Histology: reticulum cell sarcoma (reticular type).

10. Male, 63 years of age. No. 30-1223.

Initial location: neck. (Extended to shoulder girdle and chest wall.)

First symptoms developed June 1929.

Death: May 17, 1930.

Duration: 11 months.

Histology: reticulum cell sarcoma (with zones of reticulated and undifferentiated types).

11. Male, 37 years of age. No. 24-204.

Initial location: neck (widespread adenopathy).

First symptoms developed January 1923.

Death: May 25, 1924.

Duration: 1½ years.

Histology: reticulum cell sarcoma (differentiated type).

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DESCRIPTION OF PLATES

PLATE 73

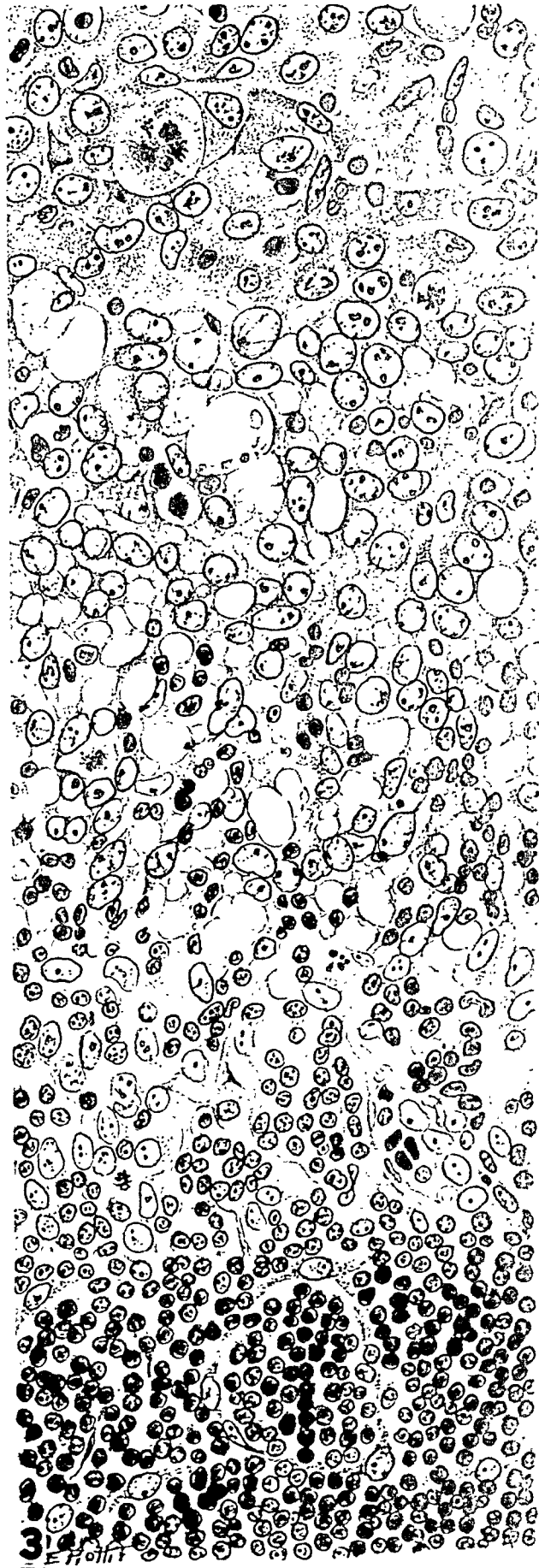
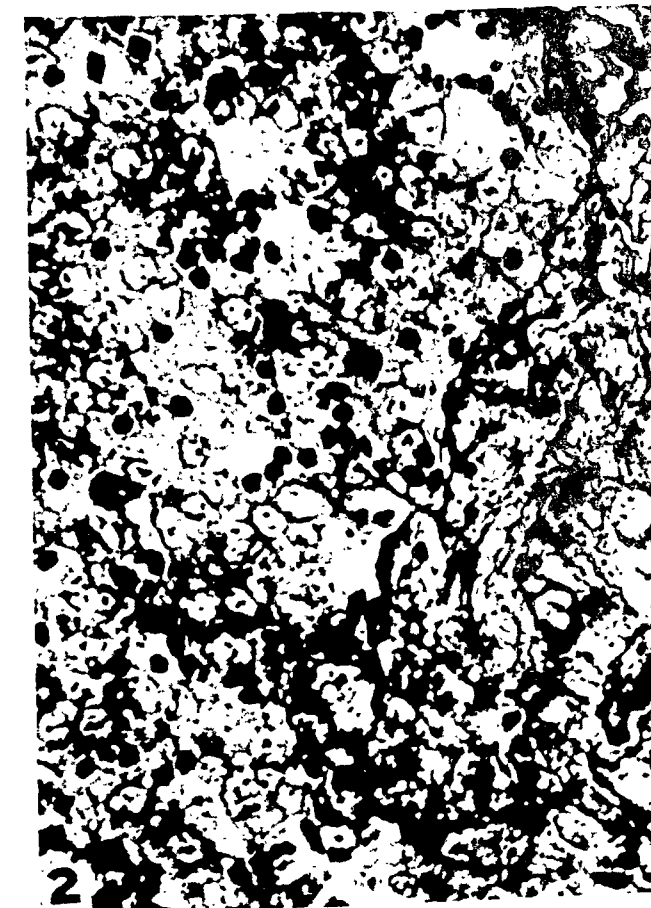
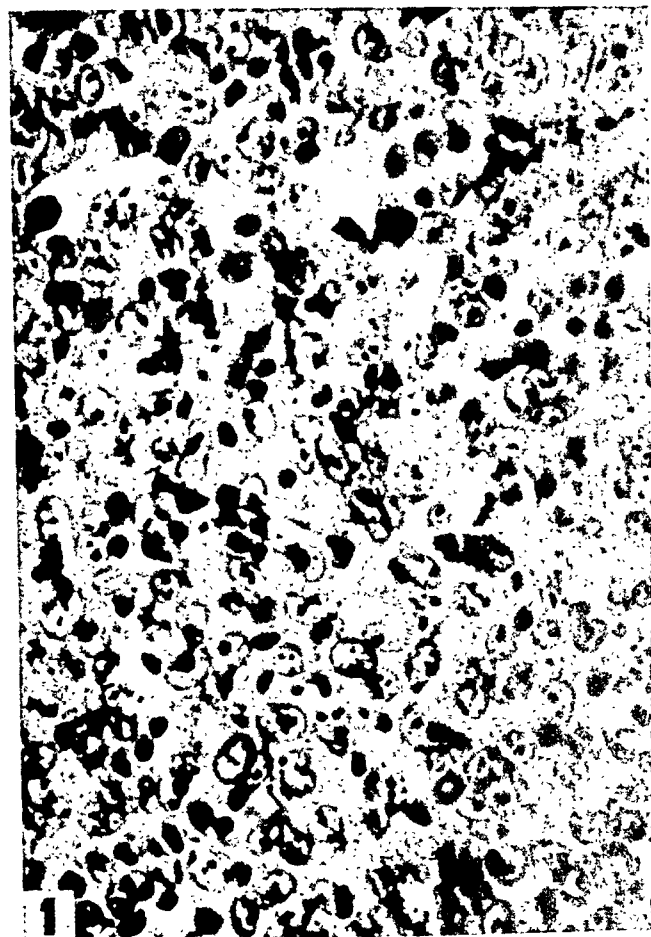
- FIG. 1. Reticulum cell sarcoma, showing syncytium. $\times 410$.
- FIG. 2. Reticulum cell sarcoma (case 1), showing syncytium and reticulum. $\times 410$.
- FIG. 3. Diagrammatic drawing taken from actual cases, showing changes in degree of differentiation from reticulum cell sarcoma of syncytial type to malignant lymphoma.

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PLATE 74

FIG. 4. Reticulum cell sarcoma (case 9), reticulated type. $\times 410$.

FIG. 5. Reticulum cell sarcoma (case 9), reticulated type, stained for reticulum. $\times 410$.



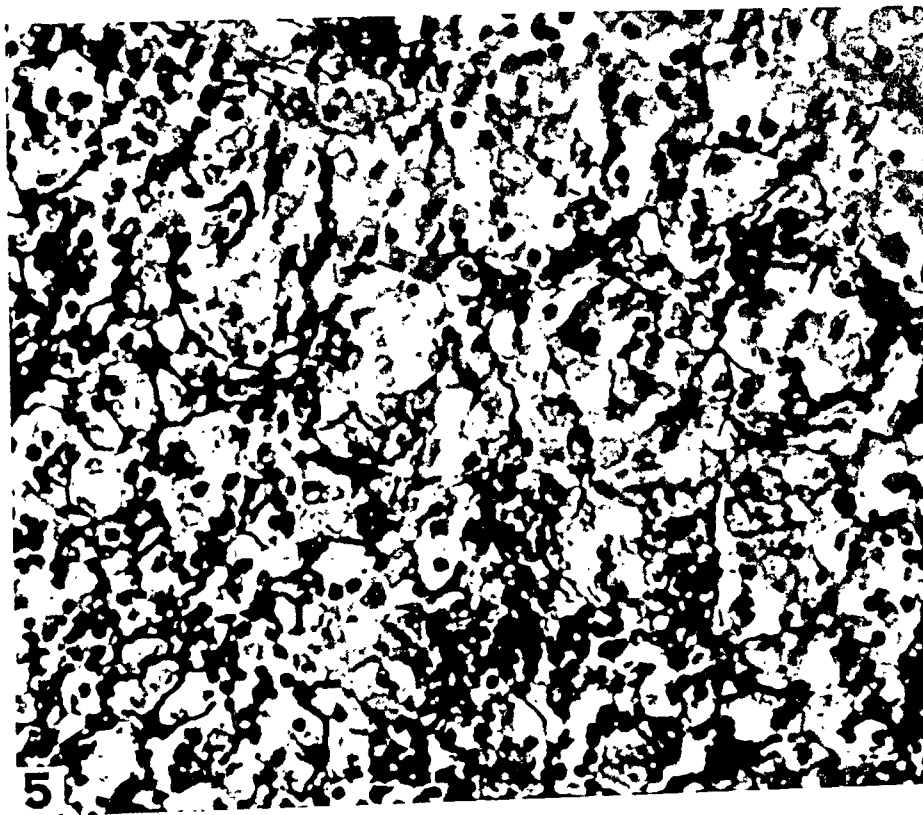
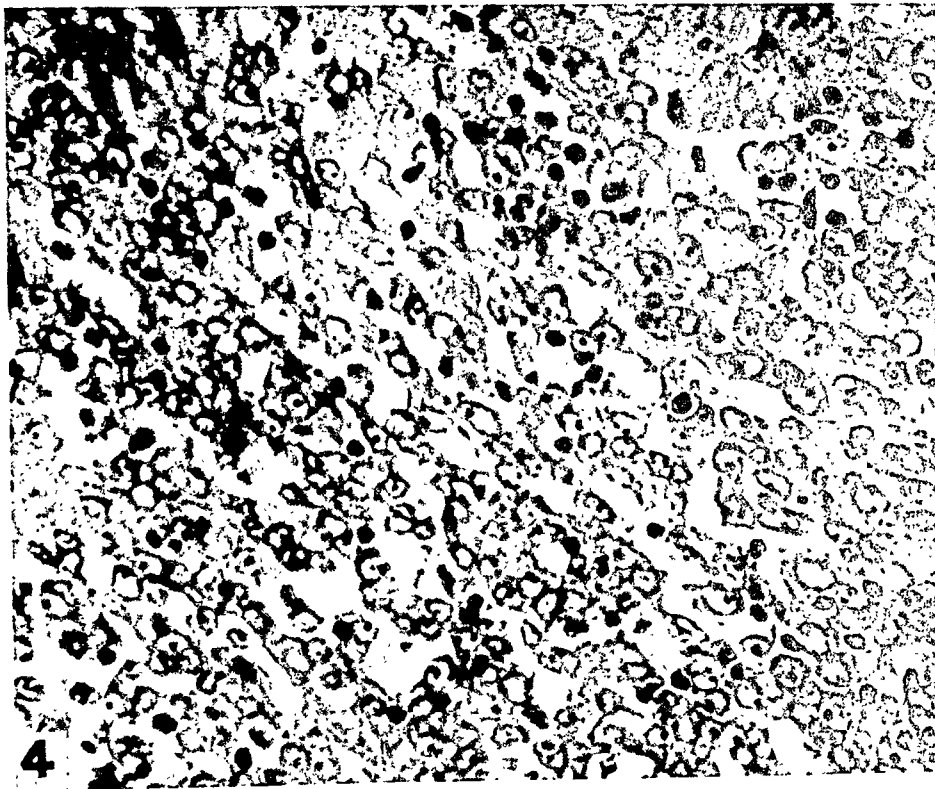
Reticulum Cell Sarcoma of Lymph Nodes

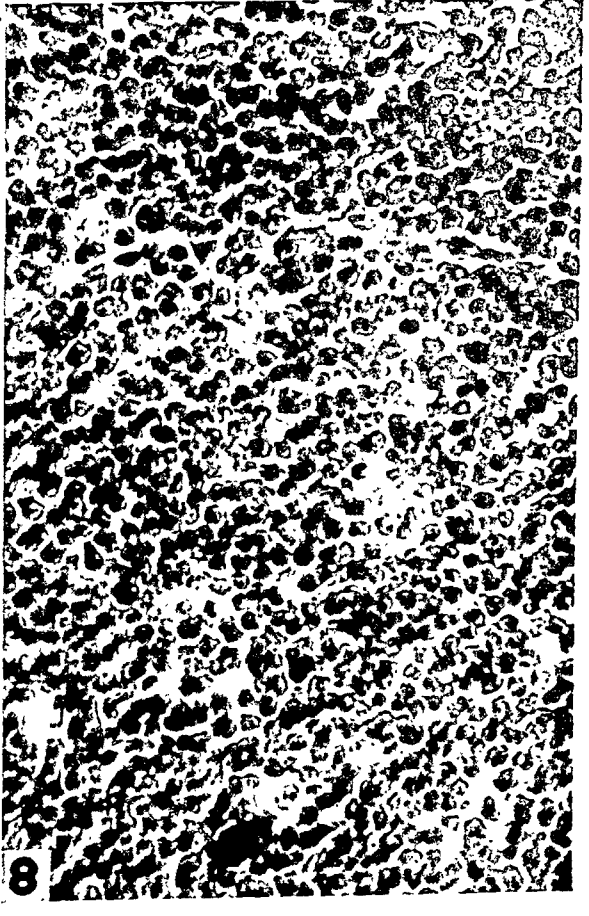
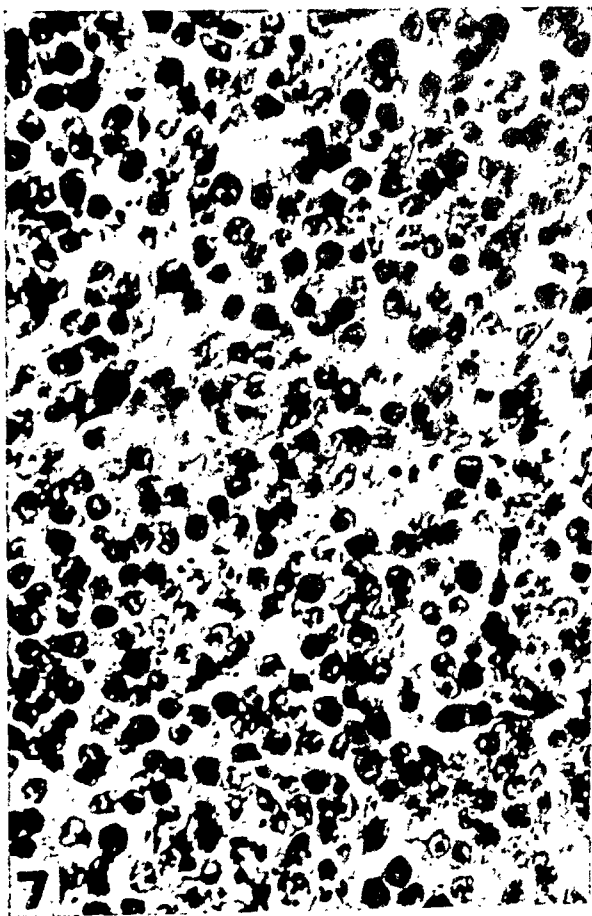
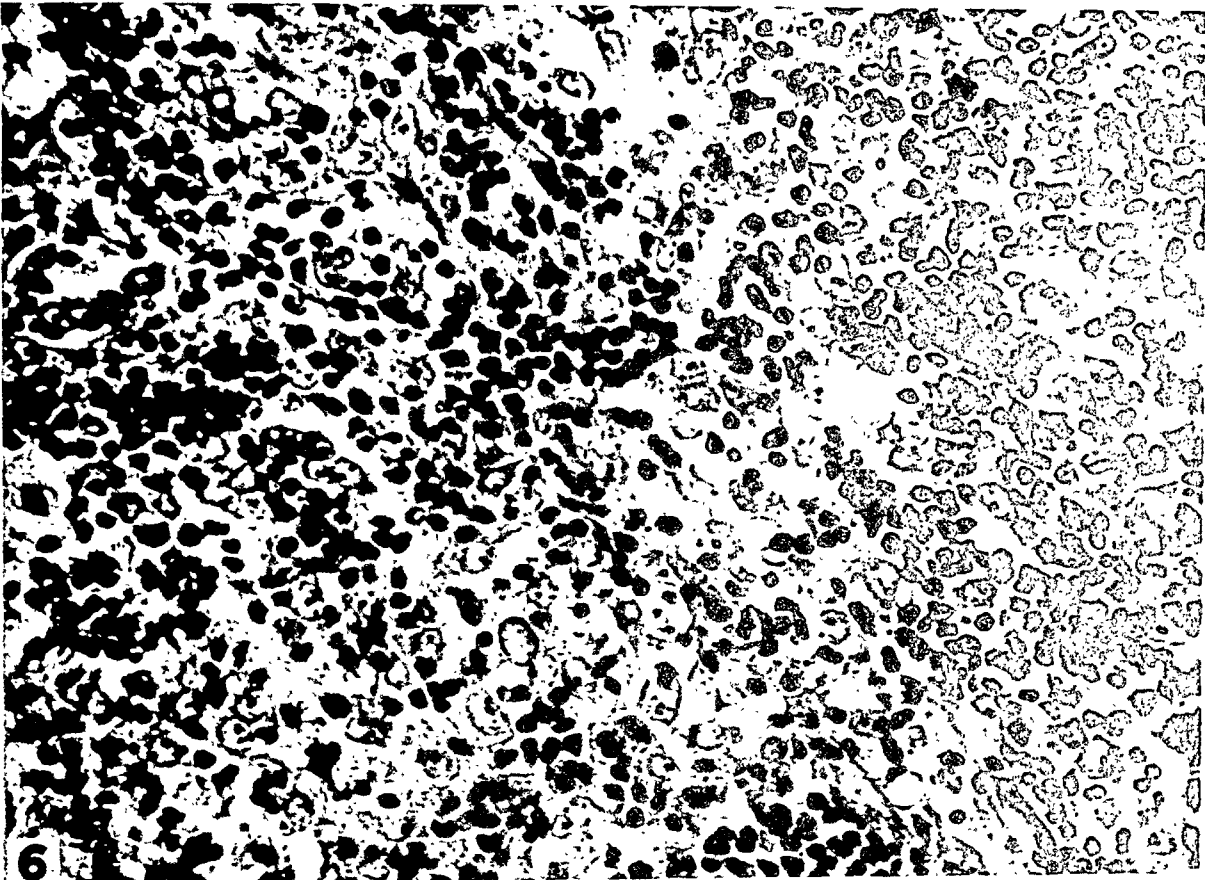
PLATE 75

FIG. 6. Reticulum cell sarcoma (case 9), mixed type. $\times 410$.

FIG. 7. Lymphosarcoma (lymphoblastoma type). No. 32-1563. $\times 410$.

FIG. 8. Malignant lymphoma. No. 39-73. $\times 410$.





obtained with the author's chromium hematoxylin-phloxin stain as late as 10 hours after death. Two islet cell adenomas were obtained from the Department of Surgery of the same hospital. Five pancreases from diabetic patients were received from the Laboratory of Pathology of the New England Deaconess Hospital in Boston. The total number of pancreases from diabetic patients was 11. The remaining 59 were from nondiabetic individuals. In this last group there were 3 with gross pancreatic lesions: benign islet cell adenoma with hypoglycemia, 2 cases; islet cell carcinoma with hypoglycemia, 1 case.

One other, rather unique, case (No. 4561) will be mentioned here because the clinical picture was that of an insulin-producing tumor of the pancreas. A man, aged 56 years, complained of dizzy spells and repeated fainting for 4 months before admission. During his stay in the hospital typical hypoglycemic attacks were observed with blood sugar values as low as 31 mg. per 100 cc. At laparotomy the pancreas was found to be normal. Radon seeds were inserted and the abdomen was closed. The patient died of shock on the next day. At autopsy the pancreas contained no neoplasm nor was there any hyperplasia of the islets present. A huge, ovoid, solid tumor, bulging from the upper aspect of the right lobe of the liver and extending deeply into the liver itself, was the only essential finding at autopsy. On microscopic examination the tumor proved to be a fibroma. Bio-assay failed to show the presence of insulin in it. The mechanism of hypoglycemia in this case is not well understood.

Of all material, slices not exceeding 2 mm. in thickness were cut and fixed in Bouin's fluid, in Bayley's modified Zenker-formaldehyde and in Stieve's fluid. After 30 minutes, when the slices were firm enough, they were halved in thickness with a razor blade and replaced in the fixing fluid for 24 hours. All fixatives proved to be quite satisfactory for the specific stains, but Bouin's fluid was found to be the best. The modified Bouin's fluid as described in the original chromium hematoxylin-phloxin technic, though it gives the sharpest differentiation of the beta cells, was not used because its penetration is rather slow, and blocks often are unevenly fixed. Fixation at ice box temperature proved of no advantage. The slices were embedded in paraffin and cut at from 2 to 4 μ .

OBSERVATIONS WITH DIFFERENTIAL STAINS ON HUMAN ISLETS OF LANGERHANS*

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Whereas reports on the number and size of the islets of Langerhans under normal, experimental and pathological conditions, and on changes observable with routine stains, would fill volumes, there is very scant literature available on observations made with the aid of specific stains, especially in respect to human material. This is due to the fact that up to 1931 the only known differential stains for the study of islet cells were the neutral stains of the type Lane and Bensley described. These stains give beautiful pictures on guinea pig material. However, in other animal species and on human material their results are much poorer and often very hard to interpret. The use of the Mallory-Heidenhain azan stain as a differential stain for islet cells was first suggested by Bloom in 1931. It can be used successfully in practically all species, but it does not give a clear picture of the beta cells which, according to most authors, must be considered the source of insulin. This may explain the fact that, as Warren stated, no attempt has been made so far to do differential counts on human islets. In 1939 I published a staining method that gives a sharp differentiation between alpha and beta cells in all species. Another specific stain for dog material was developed by Richardson in 1940.

The purpose of this paper is to give an account of observations made with specific stains on 70 human pancreases, normal and pathological.

MATERIAL AND METHODS

Most of the material came from autopsies at the Billings Hospital of the University of Chicago. Only those cases in which autopsy was performed within 4 hours after death are included in this study, although in many instances excellent results were

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in the hematoxylin solution has been decreased because with the original amount of dichromate the stain will keep its full staining power and selectivity for only about 10 days; (c) for the better staining of the alpha granules a thorough washing of the sections after phosphotungstic acid is recommended. The summary of the improved method is as follows:

1. Run sections through xylol and alcohols to water.
2. Refix in Bouin's fluid for from 12 to 24 hours.
3. Wash sections thoroughly in tap water to remove picric acid.
4. Treat sections for about 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and sulfuric acid.
5. Decolorize with a 2 to 5 per cent solution of sodium bisulfite. Wash.
6. Stain in the following hematoxylin solution under microscopic control until the beta cells stand out deep blue (about 10 to 15 minutes): Mix equal parts of 1 per cent aqueous solution of hematoxylin (National Aniline hematoxylin C. P. and Grüber's dark hematoxylin are very satisfactory) and of 3 per cent solution of chromium alum. Add to each 100 cc. of the mixture 2 cc. of 5 per cent solution of potassium dichromate and 2 cc. of N/2 (about 2.5 per cent) sulfuric acid. The mixture is ripe after 48 hours and can be used as long as a film with a metallic luster will continue to form on its surface after 1 day's standing in a Coplin jar (about 4 to 8 weeks). Filter before use.
7. Differentiate in 1 per cent hydrochloric acid-alcohol for about 1 minute.
8. Wash under the tap until the section is a clear blue.
9. Counterstain with 0.5 per cent aqueous solution of phloxin for 5 minutes. Rinse.
10. Immerse in 5 per cent phosphotungstic acid solution for 1 minute.
11. Wash under the tap for 5 minutes. The section should regain its red color.
12. Differentiate in 95 per cent alcohol. If the section is too red and the alpha cells do not stand out clearly enough, rinse the section for about 15 to 20 seconds in 80 per cent alcohol.
13. Transfer to absolute alcohol, clear in xylol and mount in balsam.

The results of the three staining methods were compared by staining each of three consecutive serial sections cut at $2\ \mu$ with a different stain. The methods were found to be completely comparable, and no overlapping of alpha and beta cells with different stains was observed. With chromium hematoxylin-phloxin the beta cells are blue, alpha cells red, D cells from pink to red and indistinguishable from alphas.) With the azan stain the beta cells have indistinct dingy grayish granules, the alpha cells ruby red granules and the D cells deep blue granules. Phosphotungstic acid hematoxylin does not stain the beta granules; the alpha granules are steel gray to blue-black; the D granules are either unstained

As neither the neutral stains nor Richardson's technic gave satisfactory results, only the following stains were used:

1. *The Mallory-Heidenhain azan stain.* It was found that the D cells will stain far sharper and darker if the staining time with azocarmine is prolonged. Optimal results were obtained by staining for 10 to 16 hours at 65° C. in a 0.05 per cent azocarmine G solution containing 1 per cent acetic acid. Differentiation in aniline-alcohol may take a rather long time after this prolonged staining, but it may be greatly shortened by adding about 10 per cent of distilled water to the differentiating fluid. Alpha cells can be brought out with extreme clarity if the sections are treated before staining for about 30 seconds with a 0.1 per cent solution of potassium permanganate and subsequently decolorized with sodium bisulfite.

2. *Mallory's phosphotungstic acid hematoxylin.* If after permanganate oxidation the sections are mordanted for about 1 hour or longer in a 2 to 5 per cent solution of iron alum and then stained in Mallory's phosphotungstic acid hematoxylin for 8 to 24 hours, depending on the type of fixation and on the thickness of the sections, very sharp pictures are obtained with excellent visualization of certain cellular details. Zymogen granules are a rusty red, alpha granules a deep steel gray, mitochondria and terminal bars blue-black. Beta granules are unstained and D granules are either unstained or a dull lilac. This stain is also recommended for the staining of other structures, such as cross striations in muscle tissue and epithelial fibers.

3. *Gomori's chromium hematoxylin-phloxin stain.* Since the original publication of this technic, attempts have been made to simplify the method and, at the same time, to make it work with a high degree of constancy of results. It was found that after a suitable pretreatment (refixation and permanganate oxidation) practically any basic stain will bring out the beta cells more or less selectively. However, only acridine red of Grübler (but not other brands of the same dye) and chromium hematoxylin were found to give perfectly clear definition. As the counterstaining of sections stained with acridine red is somewhat difficult, it is better to depend on hematoxylin. The following modifications of the technic proved to be useful: (a) Bouin's fluid is entirely satisfactory for refixation; (b) the amount of potassium dichromate

of the three cell types. Their number is roughly proportionate to that of the alpha cells. Under normal conditions they account for from 2 to 8 per cent of all islet cells. Their predilectional sites are exactly the same as those of the alpha cells. The same applies to their shape and size as well as to the size and distribution of their granules, the only difference being the special staining reaction of the D granules. Moreover, transitions between alpha and D cells are often observed. All intermediate shades from ruby red to clear blue granules can be seen in any adequately stained section of fresh, well fixed material. There may be serious difficulty in telling whether an occasional cell is more like an alpha cell or a D cell. There is some evidence, as it will be pointed out later, that D cells represent but a stage of ageing of the alpha cells.

These wide variations in the cellular composition of the islets under normal conditions cast considerable doubt on the value of reports dealing with the increase or decrease in the numbers of either beta or alpha cells under various experimental conditions.

There is great variability also in regard to the extent and degree to which the cells are granulated. Sometimes 80 to 90 per cent of all beta cells are densely packed with dustlike, dark blue granules. In most cases, however, granulation is less complete. Three different forms of degranulation of beta cells have been observed, which often occur in combination:

1. Diffuse degranulation (Fig. 3). Throughout the islet the beta granules show rarefaction. They are strewn more or less sparsely but uniformly in the cytoplasm of the cells.

2. Discontinuous degranulation (Fig. 4). Many of the beta cells in an islet are completely, or almost completely, degranulated; while others, scattered at random, are fully granulated and dark.

3. Margination (Fig. 5). Granules can be seen in the cell cords only at their walls adjoining capillaries. They occupy one side of the cell only. The central parts of the cell cords are degranulated. Under low power the lobules of the islet are pale areas, rimmed with dark blue.

Both diffuse and discontinuous degranulation has been found, though less commonly, in alpha cells also. Considerable discontinuous degranulation of both beta and alpha cells may render an

or a pale lilac. In general, neither the azan stain nor phosphotungstic acid hematoxylin will yield clear pictures if the material is older than 2 to 3 hours, whereas, as mentioned, the chromium hematoxylin-phloxin stain may give beautiful pictures even if the material is older.

OBSERVATIONS

Differential Counts on the Islets Under Normal and Pathological Conditions

Sections of each pancreas were stained with chromium hematoxylin and the Mallory-Heidenhain azan stain. Of 70 pancreases, the state of preservation in 6 was too poor for any differential stain. The azan stain was unsatisfactory in 9 more. The remaining 55 were satisfactory for complete differential counts. At least 6 islets were counted in every section, totalling from 500 to 1200 cells.

Although in some pancreases the proportion between the numbers of the different cell types is rather uniform throughout all islets, usually, even under normal conditions, the ratio is extremely variable not only in different areas, but also in the same section.* It is by no means uncommon to find an islet with a beta:alpha ratio of 6 or higher next to another islet having a ratio far below 1, *i.e.*, consisting predominantly of alpha cells (Fig. 1). Since the alpha cells have a tendency to nestle against the walls of capillaries at the periphery of the cell cords, whereas the beta cells most often occupy the more central, avascular areas of the cell cords (Fig. 2), it is a common observation that high vascularization of an islet means a high alpha count in that islet. Islets having many capillaries may consist almost exclusively of alpha cells. On the average, around 60 to 90 per cent of all islet cells are beta cells, the rest being alpha cells and D cells. In approximately 66 per cent of all cases without demonstrable pathology in the pancreas, the beta:alpha ratio was between 3 and 8; in 15 per cent between 1.2 and 3; in 19 per cent between 8 and 11. These counts having been done with the chromium hematoxylin-phloxin stain, D cells were counted as alpha cells.

The azan stain shows that the D cells are the least numerous

* The same thing was found to be true in respect to the cellular composition of the islets of the dog. This is in contrast with the findings of Hunt who found the beta:alpha:D ratio to be fixed at 75:20:5.

In case No. 4449 (carcinoma with metastases) the islets were found to be very small, poorly demarcated and the cells greatly shrunken (Fig. 9). Some of the cells could be identified as beta or alpha cells, but the overwhelming majority took an indifferent purplish stain, without recognizable granulations. In azan-stained sections a few pale blue cells were tentatively identified as D cells.

In one case (No. 4561) of *tumor of the liver with hypoglycemia*, the findings were very interesting. The beta:alpha ratio was as low as 1.13 and 22 per cent of all cells were D cells. This is the highest D ratio observed in the whole series. In some of the islets practically all of the peripheral cells were D cells (Fig. 10). Both beta and alpha cells were densely granulated.

The Acino-Insular Relationship

The acino-insular relationship has been debated practically ever since it became known that islets are of a tissue distinctly different from the acini. There are champions of the fixed nature and noninterchangeability of the two tissues once they are fully differentiated (Lane, Bensley, Ukai, Homans, Allen and others), whereas a numerous group (Saguchi, Vincent, Otani, Sergeyeva and others) claim that the relation between the acini and islets is dynamic rather than static, and that transitions from acini to islets and *vice versa* occur under functional stimuli even in adults. The specificity of the cell types is another moot point. Bensley does not believe in the possibility of transformation between cell types alpha and beta. According to Bloom the D cells possibly represent but a stage in the evolution of beta cells.

The main source of confusion is a cell type first described and erroneously interpreted as a form of transition between acinar and islet cells by Mankowski in 1902. Under normal circumstances they are present in very small numbers, but under certain pathological and experimental conditions, such as obstruction of the pancreatic ducts by neoplasms, inflammation or ligation; long-continued feeding of animals with dry fodder, and feeding with cream, they may appear in large numbers. They develop from acinar cells, and all stages of transition between a normal, zymogen-laden acinus cell and a full-fledged Mankowski cell can be observed. The typical Mankowski cell contains a rather fine, acidophilic granulation and in many respects is similar to an alpha

exact differential count impossible, because the cells have lost their distinctive characteristics. This was the case in six instances in the present series.

No observations have been made on the degranulation of D cells.

In diabetic patients the beta:alpha ratio was found often to be lower than in nondiabetics, but the small number of cases does not permit drawing any sweeping conclusions. Out of 7 cases in which it was possible to do complete counts, 4 had very low beta:alpha ratios (0.74, 0.76, 0.83 and 1.4). The rest were entirely within the limits of the normal average. The beta cells, which are preserved even in greatly shrunken, hyaline islets (Fig. 6), often are remarkably dark and densely crowded with granules. On account of the blue-staining masses of hyalin it is sometimes hard to do differential counts for D cells in azan-stained sections, but whenever it was possible (6 cases), the D cells were found to be present in normal numbers.

In three cases of *pancreatic tumor with hypoglycemia* the findings were as follows:

Case No. 4838 (benign adenoma). Pancreatic tissue, adherent to and resected with the tumor, as well as autopsy material, was examined. However, the latter was too poorly preserved for cytological studies. In the surgical specimen both beta and alpha cells were extremely degranulated and pale (Fig. 7). The beta:alpha ratio was 8. No D cells were found.

In case No. U233821 (benign adenoma), extensive resection of the pancreas having been performed, it was possible to examine material from several parts of the gland. The islets presented extreme diffuse degranulation of both beta and alpha cells. The beta:alpha ratio was 4. Not a single D cell could be found. In addition, many of the islets showed very marked hydropic changes in the beta cells, of exactly the type that is considered the most characteristic diabetic change of the pancreas (Allen, Martin). The interesting thing was that no islets with moderate changes were found. Either they were not hydropic at all or very markedly so (Fig. 8). The hydropic degeneration of the beta cells may have been due to overburdening of the islets by the large amounts of intravenous dextrose required for the control of hypoglycemic seizures.

and occasionally even bluish. Cells in the ducts, containing clear blue granulations and indistinguishable from D cells (not to be confused with blue-staining goblet cells), were observed very rarely in this series. The conspicuously low D:alpha ratio among duct cells and in young islets as compared to that found in older islets detached from the ducts suggests the possibility that D cells are nothing but aged alpha cells. No transitions between beta cells and any other cell type found in the pancreas could be observed.

In summarizing the above data it may be said that alpha cells develop from duct epithelium; D cells are probably aged alpha cells; the origin of beta cells is unknown. There is no evidence of transformation either between acini and islets or between the cell types alpha and beta, or D and beta.

Cytological Character of Tumors Causing Hypoglycemia

Two benign adenomas and one carcinoma were observed in this series. The histological structure of these tumors will not be described in detail here. The adenomas belonged to the usual highly vascularized, trabecular type; the carcinoma was a solid, rather anaplastic tumor which at some places showed abortive gland formation. With special stains no well defined cell types, as found in normal islets, could be demonstrated in any of them. The cells took an indifferent shade with all stains and contained no identifiable granules. In case No. 4838 the tumor cells showed extremely fine, dark gray, dustlike granules, some of which were rod-shaped, with phosphotungstic acid hematoxylin. Their morphology, however, was entirely different from that of normal alpha granules, being much closer to that of mitochondria.

Other Changes in the Islets Under Various Conditions

Vacuolization of beta cells was observed in 5 of the nondiabetic cases (Fig. 16). Hydropic changes were seen in the beta cells in one case of islet cell adenoma, as already mentioned. None of the pancreases from diabetic patients showed hydropic degeneration of the beta cells.

Moderate hyalinosis of the islets was observed in 2 nondiabetic patients, one dying at the age of 47 years of a pneumococcic meningitis, the other at the age of 55 years of renal insufficiency. Both had also hyaline changes in the small arteries.

cell. However, as Bensley remarked, their granules are not true alpha granules. They differ from alpha granules in that they are coarser, much less uniform in size and are never as densely packed as the latter. (This is shown in Figs. 11 and 12, reproduced by the courtesy of Sylvia H. Bensley.) It may be added that their staining reactions are also sufficiently different from those of the alpha granules to permit easy discrimination. With the neutral stains and with chromium hematoxylin-phloxin they stain exactly like alpha granules and also with phosphotungstic acid hematoxylin, provided the section was not, or not sufficiently, oxidized with permanganate before staining. However, after energetic permanganate treatment the Mankowski granules will stain exactly like zymogen granules, *i.e.*, rusty red, while alpha granules remain deep steel gray. In a well differentiated azan-stained section the alpha granules are distinctly red, whereas the Mankowski granules are orange red. The color contrast can be greatly enhanced by permanganate pretreatment, as suggested by me. In permanganate-treated azan-stained sections the alpha granules are ruby red, while the Mankowski granules are orange yellow. Transitions between Mankowski cells and alpha cells are never observed. The former go on to degeneration by vacuolization or homogenization of their cytoplasm and possibly are the precursors of the "granular cells of the acini" described by Bayley. The Mankowski cells are probably the result of some pathological interference with the secretion and discharge of zymogen.

One of the commonest findings is that of the presence of alpha cells in the acini (Fig. 13). Occasionally, also, D cells can be found at the same site (Fig. 14). Both kinds of cells conform perfectly in regard to shape, size and orientation with the cells of the acini. Their presence cannot be suspected without special stains. Although small groups of beta cells are often seen scattered throughout the acinar parenchyma, they practically never blend so perfectly with the acinar cells as do alpha or D cells. They always give the impression of being foreign bodies wedged in between individual acini.

Alpha cells are often observed also among the cells lining small ducts and in solid epithelial buds continuous with duct epithelium (Fig. 15). Most often their granules stain a very clear ruby red, but in some of these cells the granules will stain purplish

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DESCRIPTION OF PLATES

PLATE 76

All figures on this plate were stained with the chromium hematoxylin-phloxin method and photomicrographs were taken with an orange-yellow filter.

- FIG. 1. Two adjoining islets, the one at the left with a beta:alpha ratio of 10 (partly margined); the other with a beta:alpha ratio of 0.9. $\times 160$.
- FIG. 2. Typical arrangement of cell types. Light alpha cells line the periphery of the cell cords and dark beta cells occupy the more avascular central areas. $\times 160$.
- FIG. 3. Diffuse degranulation. $\times 320$.
- FIG. 4. Discontinuous degranulation with scattered dark beta cells. $\times 320$.
- FIG. 5. Margination. $\times 260$.
- FIG. 6. Hyaline islet with dark beta cells from a patient with diabetes. $\times 260$.
- FIG. 7. An islet from case No. 4838. $\times 260$.
- FIG. 8. Hydropic degeneration of beta cells in an islet from case No. U233821. $\times 520$.

SUMMARY

The cellular composition of the pancreatic islets in normal human material shows a wide variation. On the average, the beta:alpha ratio varies from 3:1 to 8:1. D cells constitute about 2 to 8 per cent of all islet cells.

Both alpha and D cells are found in relationship to the acini as well as in the epithelium of ducts. No transitions between acini and islets, or between the cell types beta and alpha, have been observed. All intermediate stages between alpha and D cells can be demonstrated.

It was impossible to identify the cells of three tumors causing hypoglycemia as of any of the normally occurring types.

Hyalinosis of the islets was present in 2 nondiabetic cases.

NOTE: The author wishes to express his appreciation to Shields Warren of the New England Deaconess Hospital, Boston, and to the members of the Department of Pathology of the University of Chicago for their kind collaboration and help in obtaining fresh material; also to L. R. Dragstedt and A. Brunschwig of the Department of Surgery of the University of Chicago for their permission to use their cases.

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PLATE 77

Figures 9 and 16 were stained with the chromium hematoxylin-phloxin method; Figures 10 and 14 were stained with the Mallory-Heidenhain azan method; Figures 11, 12, 13 and 15 were stained with the phosphotungstic acid hematoxylin method. Photomicrographs were taken with an orange-yellow filter.

FIG. 9. An islet from case No. 4449. $\times 260$.

FIG. 10. An islet from case No. 4561. Most of the peripheral cells are D cells (dark). $\times 320$.

FIG. 11. Alpha cells in an islet of a guinea pig. At this magnification the alpha granules do not show up very distinctly. $\times 800$.

FIG. 12. Mankowski cells in the acini of the same guinea pig. Mankowski granules, although much smaller than those of zymogen, show up very distinctly at this magnification. $\times 800$.

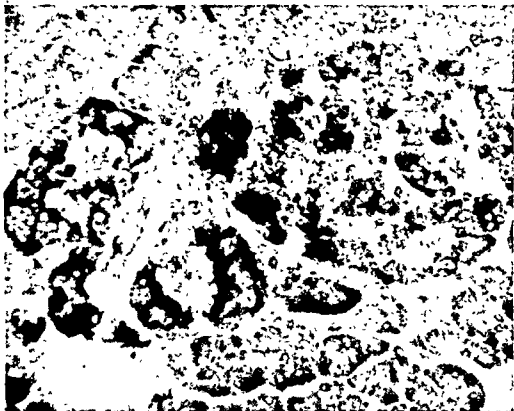
FIG. 13. Alpha cells in an acinus. To the left, the periphery of an islet. $\times 800$.

FIG. 14. A D cell in an acinus. $\times 800$.

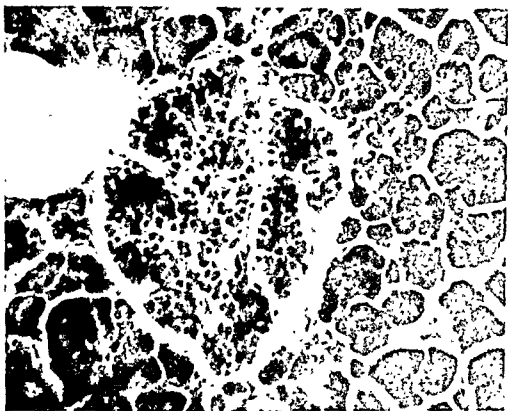
FIG. 15. Alpha cells in ducts. $\times 520$.

FIG. 16. Vacuolar changes in beta cells in the pancreas of a nondiabetic patient. $\times 800$.

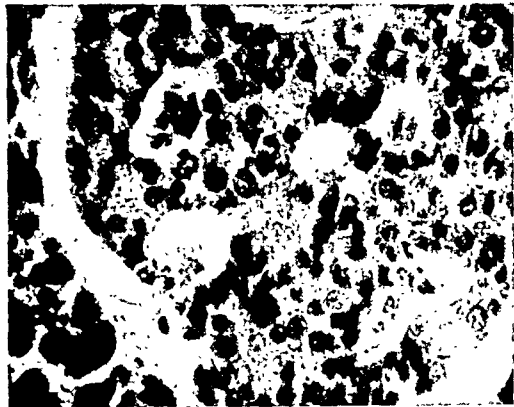
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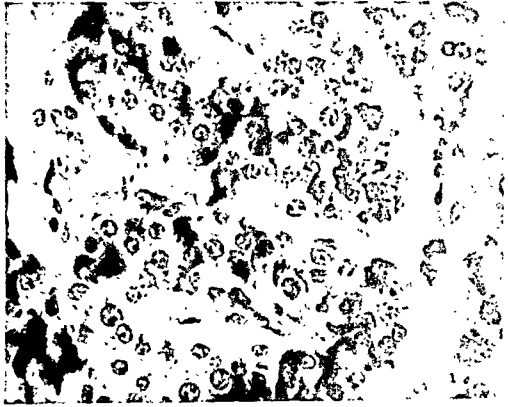
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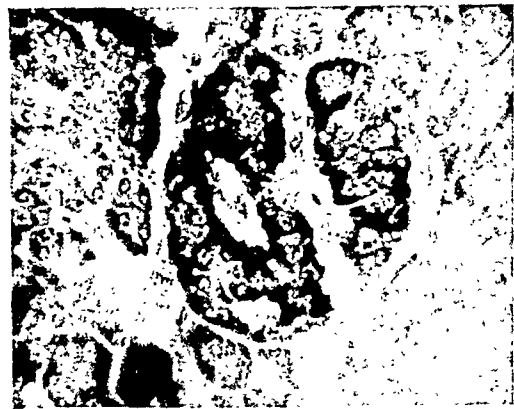
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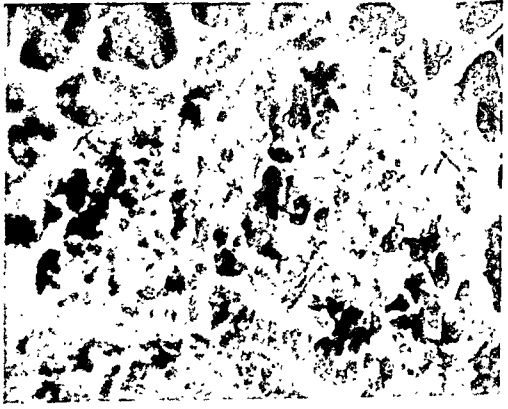
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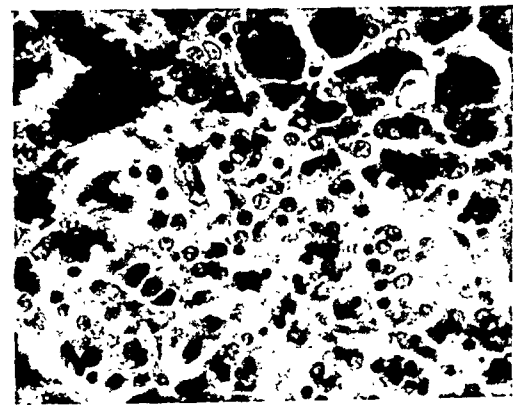
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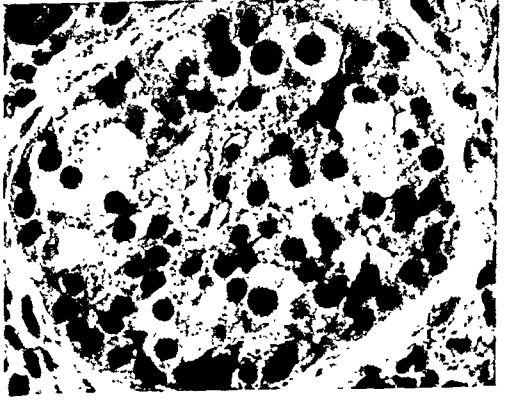
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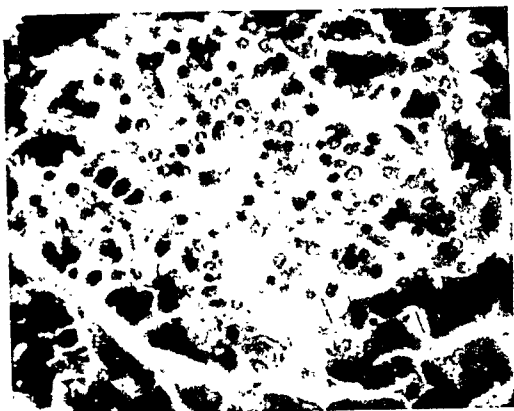
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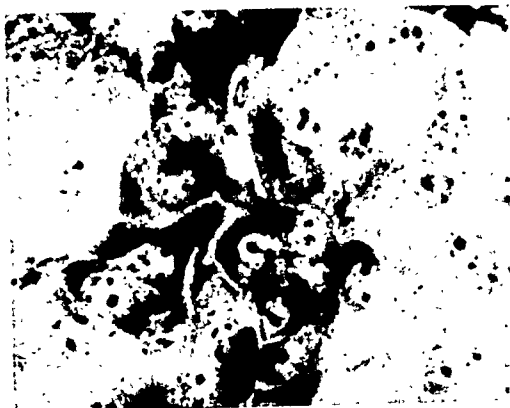
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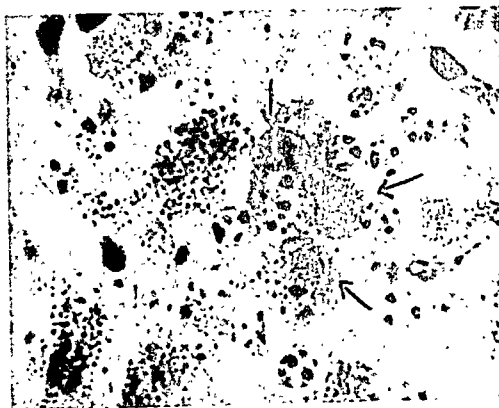
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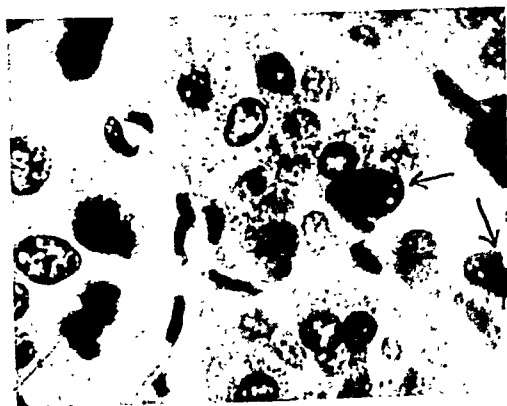
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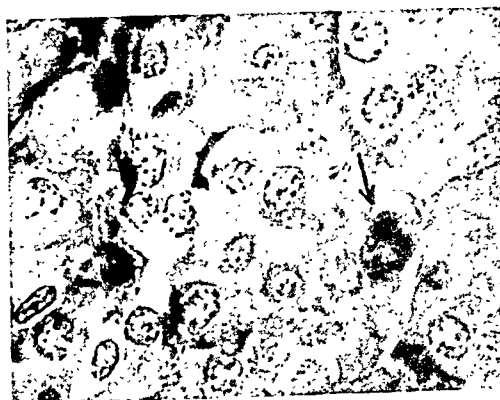
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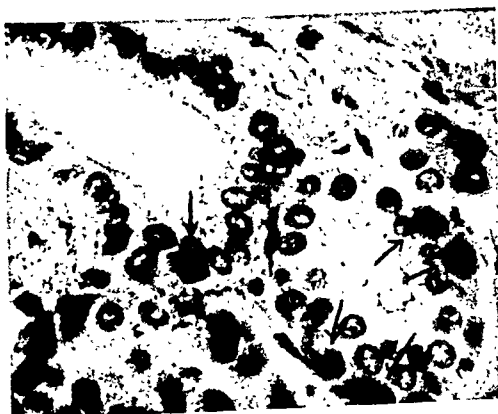
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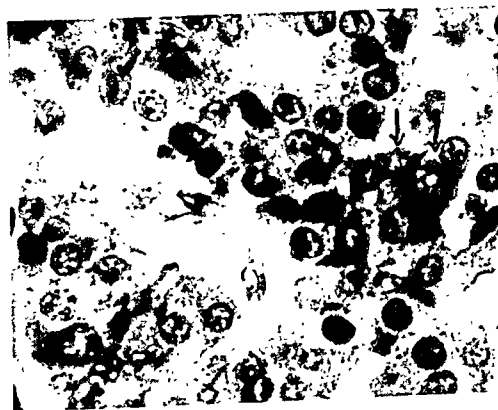
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16



and 3 days before death, had, in all instances, polymorphonuclear leukocytic pleocytosis and an increase in the protein content. For instance, the spinal fluid taken 3 days before death was described as follows: The pressure was 190 mm. of water; the fluid was cloudy and contained 1,873 cells per cu. mm., 93 per cent of which were polymorphonuclear leukocytes; protein, 156 mg.; sugar, 82 mg. and chlorides, 628 mg. per 100 cc.; colloidal gold and Wassermann reactions negative; culture showed no growth. The patient died 9 days after the onset of neurologic signs.

Postmortem Findings

At autopsy there was bronchopneumonia of the bases of the lungs. Extensive lesions were found in the white and gray matter of the cerebral hemispheres. The blood vessels were surrounded by a cuff of inflammatory cells, about half of which were polymorphonuclear leukocytes. The inflammatory reaction was marked within the walls of the vessels or confined to the perivascular spaces, but occasionally the inflammatory reaction extended into the brain tissue (Fig. 1). About half of the cells in the exudate were polymorphonuclear leukocytes, whereas the remainder were macrophages with inclusions, lymphocytes, a few plasma cells, glial cells and gitter cells (Fig. 2). There was some variation in the type of inflammatory cells in different regions. Some vessels were surrounded only by mononuclear cells, apparently lymphocytes. In some places the majority of the cells surrounding vessels were gitter cells. In sections stained for myelin sheaths there were areas of perivascular demyelination (Fig. 3) similar to those found in equine encephalomyelitis and postinfectious encephalomyelitis (postmeasles or postvaccinal encephalitis). Observations in the literature and my own experience indicate that in postinfectious encephalitis extensive involvement of the brain in the nature of perivascular demyelination and infiltration is also accompanied by a similar involvement in the spinal cord. In this case sections of the spinal cord stained by myelin sheath and cellular stains were normal. There was no perivascular infiltration and no areas of demyelination. In addition, there was only moderate involvement of the cerebellum, consisting chiefly of hyperemia. Only occasionally could polymorphonuclear leukocytes be found around some vessels and within the cerebellar tissue. These latter observations coincide with those of Wesselhoeft, Smith and Branch⁴ and Farber, Hill, Connerley and Dingle⁵ in cases of equine encephalitis in which

EQUINE ENCEPHALOMYELITIS IN MAN*

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In a previously published review¹ of 100 cases in which the diagnosis of encephalitis had been made at the Boston City Hospital between the years 1928 and 1938, one unusual case was encountered. The pathological picture in this patient, who died in 1936, was so strikingly similar to that seen in the epidemic of eastern equine encephalomyelitis which occurred in the summer of 1938 in Massachusetts,^{2,3} that it was considered advisable to report this case in detail.

The pathological picture of equine encephalomyelitis in man is so typical that it is thought to be specific.⁴ The outstanding features are an extensive inflammatory reaction, with polymorphonuclear leukocytes in the perivascular spaces and diffusely throughout the nerve tissue, and destruction of the ganglion cells, as shown by severe degenerative changes in these cells with neurophagia. The polymorphonuclear leukocytic inflammatory reaction is present even in those patients who die several or many days after the onset of symptoms. This differentiates the pathological picture from that of encephalitis lethargica, postinfectious encephalitis, acute anterior poliomyelitis, and the St. Louis type of encephalitis, in which diseases a few polymorphonuclear leukocytes may be present in the early stages of the disease but are rapidly replaced by lymphocytes and glial cells.

REPORT OF CASE

The patient was a white male, 23 years old, who died in April 1936 after an illness of 21 days. He entered the Boston City Hospital with vague complaints of chills and pains of 11 days' duration. Bronchopneumonia was suspected and X-rays of his lungs showed a patch of parenchymal infiltration at the base of the right lung. Pneumococci, type VII, were cultured from the sputum. Two days after entry the patient complained of severe headaches. The significant findings on neurologic examination were stiffness of the neck, right hemiparesis with Babinski sign and bilateral ankle clonus. The temperature ranged around 100° F. The spinal fluid, taken 5, 4,

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DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Infiltrations around vessels and extending into the brain substance of the parietal lobe. Nissl stain. $\times 100$.

FIG. 2. Perivascular infiltration around a vessel in the white matter of the parietal lobe. About half of the cells are polymorphonuclear leukocytes, whereas the remainder are macrophages with inclusions, gitter cells, lymphocytes, plasma cells and glial cells. Nissl stain. $\times 400$.

the spinal cord and cerebellum were not at all or only moderately involved.

Most of the lesions in the case here reported were within the white matter. But the gray matter also was involved. In scattered regions of the cerebral cortex capillaries were surrounded by polymorphonuclear leukocytes and in adjacent sections accumulations of glial cells and polymorphonuclear leukocytes could be observed around injured ganglion cells (Fig. 4).

Cultures of the nerve tissue and heart's blood were sterile. The spinal fluid, by culture and by intraperitoneal inoculation of guinea pigs, gave negative results.

COMMENT

This material presented a pathologic picture which could not be differentiated from that of equine encephalomyelitis in man. No attempt was made to isolate the virus. Therefore, one cannot be sure that this was a case of equine encephalomyelitis. The other possibility is that this case falls into the group of postinfectious encephalitides and the etiology may be related to the bronchopneumonia. But, as has been pointed out, the nature and distribution of the infiltrations is unlike that seen in encephalitis following such infections as measles, and the encephalitis accompanying or following pneumonia is usually a "toxic encephalitis" without any inflammatory reaction or myelin destruction.

It is not known why certain epidemic diseases lie dormant for many years and then suddenly attack the population. For this reason it seems to be important to trace sporadic cases, as this one might well be, with the hope of gaining some insight into the latency and outbreak of an epidemic.

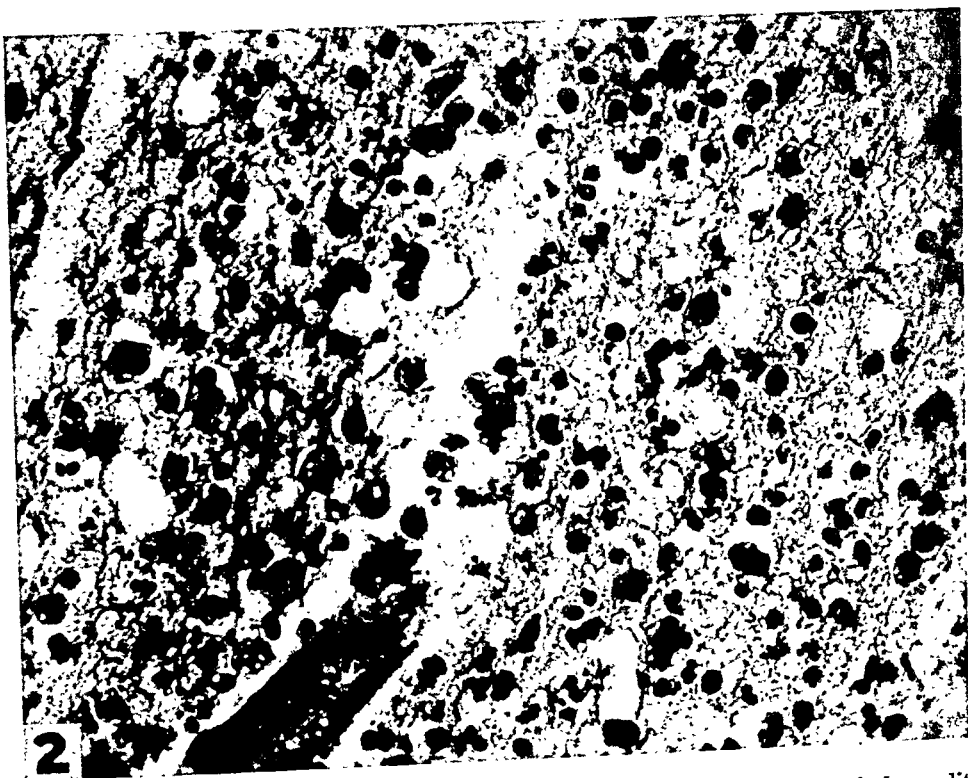
SUMMARY

A case is reported with a clinical, serologic and pathologic picture which could not be differentiated from that of equine encephalomyelitis in man. No attempt to isolate the virus of equine encephalomyelitis was made. Therefore, a definite differential diagnosis between sporadic equine encephalomyelitis and postinfectious encephalitis cannot be made, though the observations favor the former diagnosis.

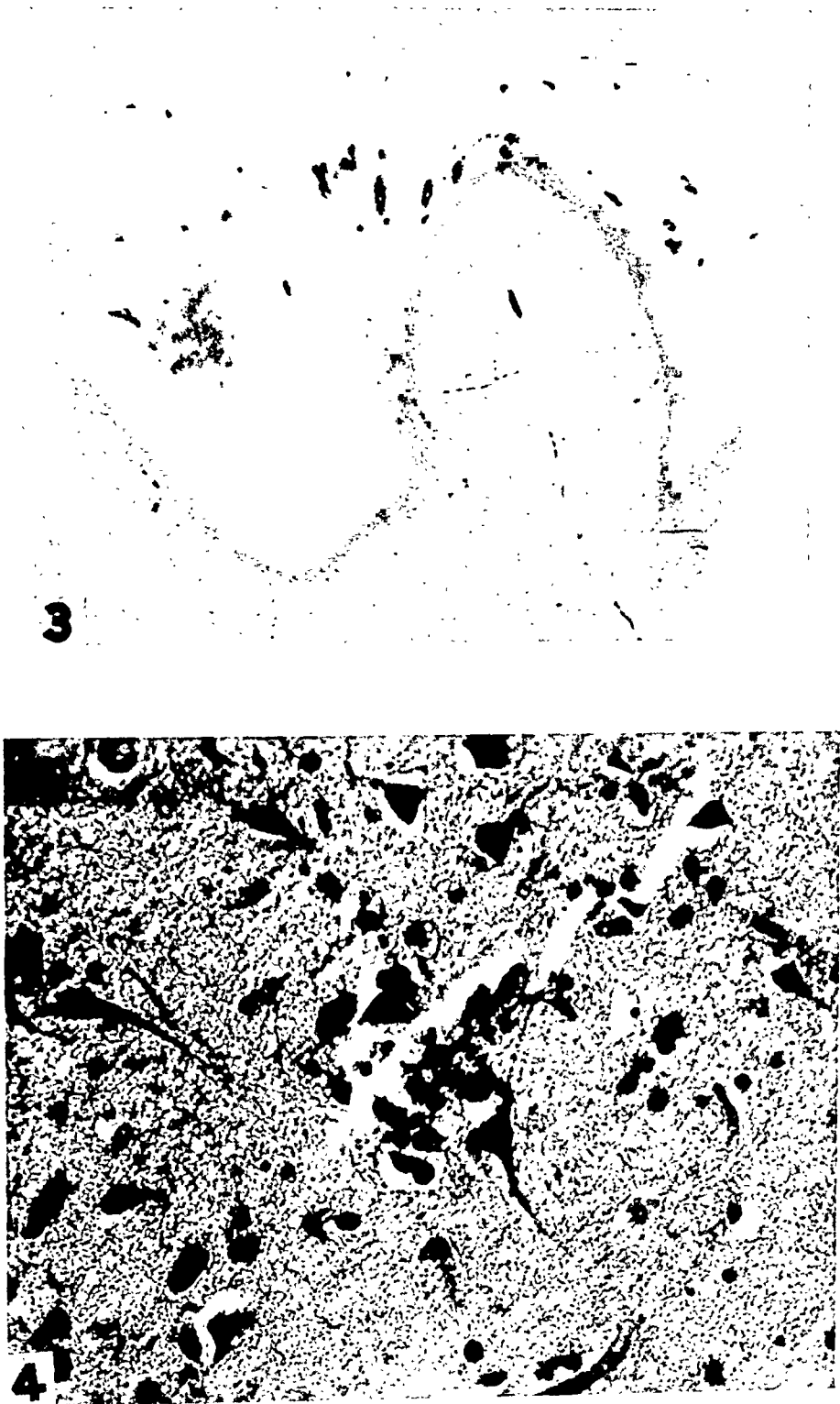
PLATE 79

FIG. 3. White matter of the frontal lobe. Areas of perivascular demyelination, similar to those observed in postinfectious encephalitis. Weigert stain. $\times 5$.

FIG. 4. Precentral cortex. Accumulation of polymorphonuclear leukocytes and glial cells around altered ganglion cells of the fourth and fifth cortical layer. Hematoxylin and eosin stain. $\times 400$.



Equine Encephalomyelitis in Man



experiments. Forty-eight hours after inoculation, embryos were fixed *in toto* in Zenker's fixative (10 per cent acetic acid). The bacteria were demonstrated in sections after staining by Wright's method, as described by Goodpasture and Anderson.² Sections were also stained with hematoxylin and eosin for routine examination. Smears and cultures were made from the amniotic fluid 48 hours after inoculation.

For the purpose of studying the lesions in the nonimmunized host, 0.1 cc. of a 24-hour broth culture was injected into the amnion of 185 embryos of 16 days' incubation. At the end of 24 hours 124 were alive; 60 were alive at the end of 48 hours; none survived 72 hours. Twenty live embryos were fixed for microscopic study at the end of 48 hours. Smears and cultures from the amniotic fluid of these were positive for *C. diphtheriae*.

In order to study the effect of passive immunization on the infection, 64 embryos of 15 days' incubation were injected subcutaneously with 0.1 cc. of commercial diphtheria antitoxin. This amount equals about 250 units. Twenty-four hours later 0.1 cc. of a 24-hour broth culture was injected into the amniotic fluid of each of these embryos. Sixty-one survived 24 hours; 55 survived 48 hours; 14 were sacrificed at 48 hours for microscopic study; 30 survived 72 hours; 24 survived 96 hours; on the fifth day 16 embryos hatched and 8 were dead. As a control, 64 embryos of 16 days' incubation which received no antitoxin were given the same amount of culture by the same route. Forty-four of these survived 24 hours; 20 survived 48 hours, and 8 were sacrificed at 48 hours for microscopic study. The remaining 12 were dead at 72 hours. Smears and cultures from the amniotic fluid of the protected and unprotected embryos which were sacrificed at 48 hours were positive for *C. diphtheriae*.

To demonstrate serial passage of the infection, embryos of 14 to 16 days' incubation were used. Into the amnion of 8 embryos 0.1 cc. of a 24-hour broth culture was injected. Forty-eight hours later another group of 8 embryos was inoculated with 0.1 cc. of amniotic fluid from 2 live embryos of the first group. This procedure was repeated for five serial passages. Smears and cultures made at the end of 48 hours from the amniotic fluid of 2 or 3 live embryos of each group were positive for *C. diphtheriae*. No embryo used in this experiment survived 72 hours.

INFECTION OF NORMAL AND PASSIVELY IMMUNIZED CHICK EMBRYOS WITH *CORYNEBACTERIUM DIPHTHERIAE**

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The use of the developing chick embryo as an experimental host for the study of bacterial infection was suggested by Goodpasture¹ in 1933. With Anderson² he demonstrated that *Corynebacterium diphtheriae* could be propagated on, and could induce a characteristic infection in, the chorio-allantois. Subsequent work by several investigators^{3,4} in this laboratory has shown that for establishing some infections the embryo could be more directly approached by way of the amniotic fluid. The mouth and nasopharynx were in this way available for portals of entry. It appeared likely that *C. diphtheriae*, introduced into the amniotic fluid, would offer a new approach for the study of this infection with especial reference to the effect of passive immunization. This report will be concerned with observations made according to this plan.

EXPERIMENTAL PROCEDURE

The strain of *C. diphtheriae* used in these experiments was isolated at autopsy from a human case of diphtheria. Typical gross and microscopic changes of diphtherial infection with membrane formation in the pharynx, larynx, trachea and bronchi were present. Unprotected guinea pigs inoculated subcutaneously with 0.5 cc. of a 24-hour broth culture of this organism succumbed within 3 days. Other guinea pigs which had previously been passively immunized with diphtheria antitoxin survived following the injection of the same amount of culture.

The inoculum used in the chick embryo experiments consisted of 0.1 cc. of a 24-hour infusion broth culture of this organism. The cover-slip technic for preparing the chick embryos as described by Goodpasture and Buddingh³ and the method of inoculating the amniotic fluid and body wall of the embryos as described by Polk, Buddingh and Goodpasture⁴ were employed. Embryos of 15 to 16 days' incubation were used throughout these

* Received for publication October 12, 1940.

small masses of bacilli were seen in the exudate. There was necrosis of many of the epithelial cells lining the parabronchi and air capillaries. Areas of necrosis and acute inflammation were seen also in the interstitial tissue of the lungs. There were areas of necrosis in the epithelium of the abdominal air sacs and a small amount of necrotic cellular exudate was present within them.

Digestive System. Small masses of bacilli were found in the oral cavities unassociated with any cellular exudate. The mucosa of the mouth and pharynx showed necrosis of a few cells but no areas of ulceration. The necrotic cells were more numerous in the basal layer of the stratified epithelium. In each of the embryos, lesions of the esophagus were seen which consisted of necrotic cellular exudate and small masses of bacilli in the lumen, areas of necrosis in the mucosa and congestion of the submucosa. Large masses of bacilli were found in the lumina of both the proventriculus and the gizzard. As in the other organs, a few phagocytes were seen among the masses of organisms and a small number of the bacilli appeared to be within the cytoplasm of these necrotic cells. There was superficial necrosis of the mucosa of the stomach and the large compound glands of the proventriculus showed focal areas of necrosis. No lesions were seen in the liver and intestines.

Circulatory System. Foci of necrosis were present in the myocardium. In these areas the nuclei were pyknotic, the homogeneous cytoplasm of the muscle fibers stained pink, and the stroma was infiltrated by monocytes and a few polymorphonuclears. No organisms were seen in these lesions. No lesions of the large blood vessels were found.

Bones. The only lesions of the bones were those which occurred adjacent to the involved cranial sinuses. These apparently represented an extension of the destructive process from the sinuses into the bone. No lesions were found in the sections of the long bones or the vertebrae.

The *spleen, adrenals* and *nervous system* showed only occasional necrotic cells. The necrotic nerve cells were found in the ganglia of the cranial nerves, none being noted in the central nervous system. The other organs showed no microscopic lesions.

PATHOLOGICAL OBSERVATIONS

Gross examination of the embryos fixed for microscopic study showed no evidence of hemorrhage or necrosis in any of the organs.

Lesions in the Nonprotected Embryos

The microscopic sections from the 20 embryos fixed 48 hours after inoculation showed the following lesions:

Respiratory System. Clumps of bacilli were seen scattered throughout the nasal passages. A few necrotic or degenerating large monocytes and polymorphonuclear cells were found among the organisms. Some of the organisms were within the cytoplasm of these necrotic cells, but the majority were extracellular. The nasal mucosa adjacent to the bacilli showed lesions which varied from small areas of focal necrosis to large areas of ulceration. An inflammatory exudate consisting of large mononuclear cells, red blood cells and polymorphonuclear cells had replaced the destroyed epithelium. With a few exceptions the cells of this exudate appeared necrotic. The submucosa adjacent to the ulcerative lesions was infiltrated by monocytes and polymorphonuclear leukocytes. The blood vessels of the submucosa were markedly congested and areas of necrosis were seen. All of the embryos showed an inflammatory reaction of varying severity within the cranial sinuses. In some of them only focal areas of necrosis of the lining epithelium were noted. In others there was complete destruction of the epithelium, infiltration of the sinuses with an exudate similar to that seen in the nasal passages, and extension of the process into the bony wall of the sinuses with necrosis of bone tissue, which was infiltrated by an acute inflammatory exudate. A few extracellular bacteria were seen in the sinuses. The lesions found in the trachea of each embryo were similar to those of the nasal passages, consisting of masses of bacilli and a few necrotic phagocytes in the lumina, areas of ulceration of the epithelium and an infiltration of the submucosa by an acute inflammatory exudate. The primary bronchi showed the same type of change as seen in the trachea. An extensive pneumonia was noted in the lungs. The parabronchi and air capillaries were filled with an exudate of monocytes, polymorphonuclears and red blood cells. Most of the cells of this exudate appeared necrotic, and

a few had undergone necrosis. There was no evidence of injury to the mucosa.

There were no areas of focal necrosis in the myocardium. No necrotic foci were seen in the spleens, adrenals, nervous systems, lungs or bone marrow of the protected embryos.

DISCUSSION

The scope of this report does not include a review of the numerous and extensive experimental studies performed with *C. diphtheriae* and its toxin in various experimental animals. A very complete review of this work was made by Andrewes, Bulloch, Dreyer, Gardner, Fildes, Ledingham and Wolf⁵ in 1923. Past researches show that the mere application of cultures of *C. diphtheriae* to the intact skin or mucous membrane of experimental animals is rarely if ever capable of inducing experimental diphtheria. A previous injury to the surface is apparently essential and even under these circumstances it is not possible to carry on a series of passages, apparently because the microbes rapidly diminish in number after being introduced into the usual experimental animal. That this infection can be induced in serial passage from embryo to embryo was shown by Goodpasture and Anderson² who carried the infection for ten generations on the chorio-allantoic membrane. Serial passage of the infection can also be carried out when the embryos are infected by subamniotic inoculation.

Because of the sensitivity of the guinea pig to diphtheria toxin, our knowledge of the factors involved in the toxin-antitoxin reaction is quite extensive and adequate. We have, however, little experimental demonstration of the action of the microörganism at the sites of predilection where it is capable of producing the disease.

Following subamniotic inoculation, the susceptible chick embryo acquires the infection by means not very unlike those under natural circumstances in the usual human host, *i.e.*, by way of the mouth and nasopharynx. In this completely susceptible host the organism thrives in the fluids bathing the mucous membranes of the mouth, nasopharynx, trachea, lungs, esophagus and stomach. Sufficient toxin is apparently liberated to produce necrosis of the mucosa without a predisposing injury.

Lesions in Passively Immunized Embryos

Respiratory System. In the nasal passages a few large mononuclear cells were found with an occasional rod-shaped bacterium in the cytoplasm of some of these cells. There was no necrosis of the nasal epithelium and no infiltration of the submucosa by an inflammatory exudate. The cranial sinuses contained a moderate amount of exudate, consisting chiefly of large mononuclear cells and a few polymorphonuclear leukocytes. The cells of this exudate showed no evidence of necrosis. In the cytoplasm of a few of the large monocytes were small clumps of bacilli. No extracellular bacteria were seen in the sinuses. The mucous membrane lining the sinuses was everywhere intact and the adjacent bony structures showed no necrosis. The tracheae contained a varying amount of exudate but in none of the sections was there any evidence of ulceration of the tracheal epithelium. In some of the embryos the entire lumen of the trachea was filled with an exudate consisting chiefly of large mononuclear cells. The cells were well preserved and many of them contained numerous bacilli in their cytoplasm. A similar reaction was seen in the bronchi. The lungs showed scattered areas of pneumonia. No necrosis of the parabronchial epithelium or any interstitial reaction was noted. The exudate in the parabronchi and air capillaries was of the same type as found in other portions of the respiratory tract. The cells were well preserved and a few of them contained bacilli. The abdominal air sacs contained a small amount of exudate but showed no necrosis or ulcerative lesions of the lining epithelium.

Digestive System. In the oral cavity and pharynx an occasional mass of bacilli was present. There was no cellular reaction and no evidence of injury to the mucosal lining of these passages. No ulcerative lesions were found in the esophagus of any embryo studied. Occasionally a small amount of cellular exudate composed chiefly of monocytes was present in the lumen.

Both the proventriculus and the gizzard of each embryo contained a large amount of cellular exudate and a few masses of bacilli in their lumina. This exudate also consisted chiefly of large monocytes and a few polymorphonuclear leukocytes. Many of the monocytes had phagocytosed small clumps of bacilli. Only

ceptible to phagocytosis, but the phagocytes remain viable and are able to perform their function in the absence of toxin. Hammerschmidt,⁶ who investigated the reaction to the growth of *C. diphtheriae* in a nodule of nutrient agar introduced subcutaneously in guinea pigs, observed that phagocytosis of the organisms by large monocytes in passively immunized animals is an important factor in reducing the rate of proliferation of the organisms. He was of the opinion that neither bactericidal substances nor opsonins played an important part in resistance to the infection under these circumstances.

In the chick embryo these well-recognized factors in the pathogenesis of diphtheria are subject to demonstration in a way until now not possible in any other experimental animal. Some of the factors concerned in the mode of action of diphtheria antitoxin are clearly emphasized. This host was used by Buddingh and Polk⁷ to assay meningococcus antisera and also to analyze certain factors concerned in the protection conferred by these substances against infection by the meningococcus. Since a modified lesion may be produced in chick embryos by the use of therapeutic agents such as diphtheria antitoxin, the possibility of using this host for studying the mode of action of chemotherapeutic agents is suggested.

SUMMARY

1. The developing chick embryo is susceptible to infection with *C. diphtheriae* when the mouth and nasopharynx serve as a portal of entry. The resulting lesions simulate those of the natural infection in man. Diphtherial infection of chick embryos can be carried on in serial passage when the infection is induced by subamniotic inoculation.

2. Some of the factors concerned with pathogenesis of diphtheria and with the protective mechanism produced by the administration of antitoxin are subject to analysis by a comparative study of the lesions found in unprotected and in passively immunized embryos.

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It is of importance to note that the essential characteristics of the disease as it occurs in the natural host, man, are reproduced in the developing embryo. Superficial growth of the bacillus without invasion takes place. The diffusion of toxin produces necrosis and this in turn enhances the growth-promoting character of the environment. Absorption of the toxin into the blood stream takes place and necrosis in the myocardium, adrenals, spleen and peripheral ganglia results from its selective pharmacological effect upon specific types of tissue.

It is unusual for the organism to produce a lesion in the stomach of the natural host, apparently because the gastric juice is inimical to the growth of the bacillus, but in this experimental host abundant growth occurs in the gastric fluids and lesions of the mucosa are produced. The development of the lesion does not depend primarily on the presence of a *locus minoris resistentiae* but on the availability of suitable environmental and growth factors.

These factors concerned in the pathogenesis of diphtherial infection are further emphasized in the passively protected embryo. It is significant in the first place that the antitoxin persists in the embryo as it does in other animals. The establishment of the disease in the unprotected host depends on the production of sufficient toxin. This, by its injurious effect, is responsible for the necrosis of the adjacent mucous membrane and the ensuing inflammatory reaction. The degenerating exudate apparently provides an excellent medium for the further rapid proliferation of the microorganism and increasing toxin production. In the passively immunized animals the neutralization of the toxin modifies the infection markedly. There is a less suitable environment for growth, and proliferation progresses at a reduced rate. The presence of the secretions of the intact mucous membranes, the absence of necrotic tissue and serum, and the phagocytosis of the organisms by large mononuclear cells which remain viable, are the important factors which result in the reduced rate of proliferation of the bacteria. The possibility that some antibacterial factor of the nature of opsonins or tropins may be supplied by the antitoxic serum must be considered, but such an assumption does not seem to be necessary to explain the alteration of the infectious process. The bacteria are not necessarily more sus-

DESCRIPTION OF PLATES

PLATE 80

- FIG. 1. Cranial sinus of nonimmunized chick embryo, infected with *Corynebacterium diphtheriae*, showing necrotic exudate and necrosis of mucous membrane and adjacent bone. Hematoxylin and eosin stain. $\times 120$.
- FIG. 2. Lung of nonimmunized chick embryo infected with *Corynebacterium diphtheriae* showing areas of necrosis in the lung tissue and necrotic exudate. Hematoxylin and eosin stain. $\times 120$.
- FIG. 3. Toxic necrosis in myocardium of nonimmunized chick embryo infected with *Corynebacterium diphtheriae*. Hematoxylin and eosin stain. $\times 550$.

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PLATE 81

- FIG. 4. Lung of unprotected chick embryo showing necrotic exudate, necrosis of lung tissue, and masses of diphtheria bacilli. Hematoxylin and eosin stain. $\times 700$.
- FIG. 5. Lung of chick embryo, passively immunized with commercial diphtheria antitoxin, showing a monocytic exudate and minimal evidence of necrosis following infection with *Corynebacterium diphtheriae*. Hematoxylin and eosin stain. $\times 700$.

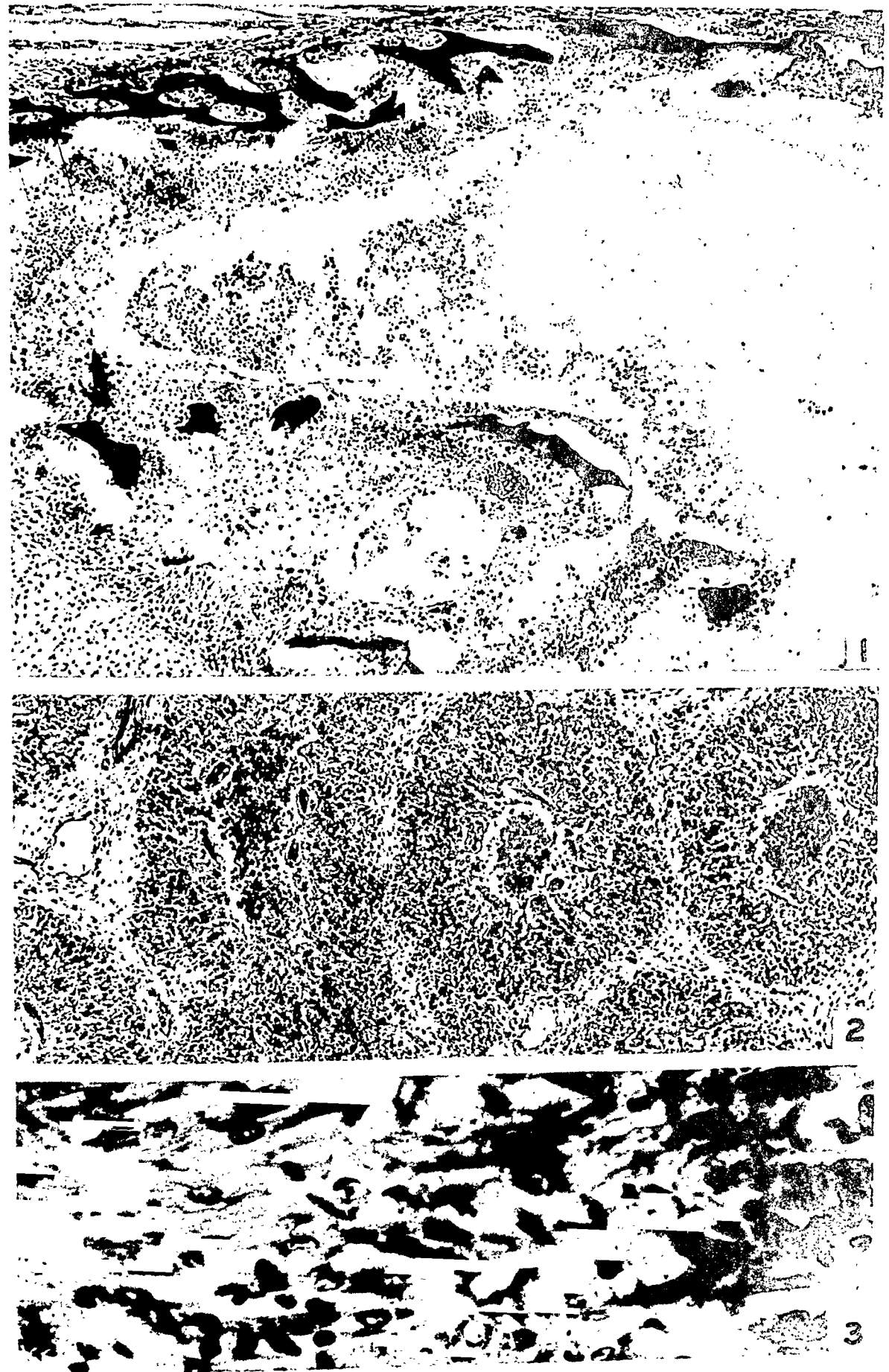
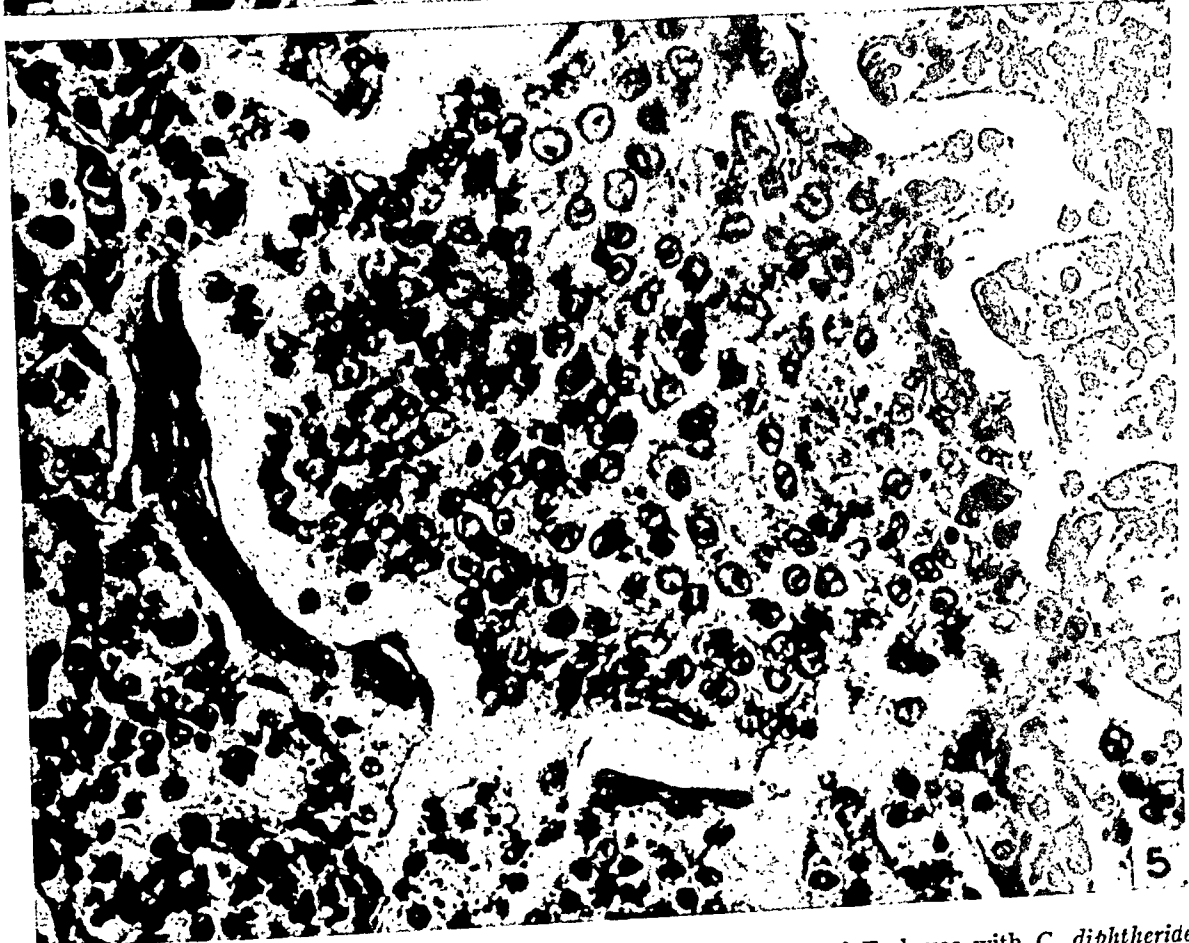
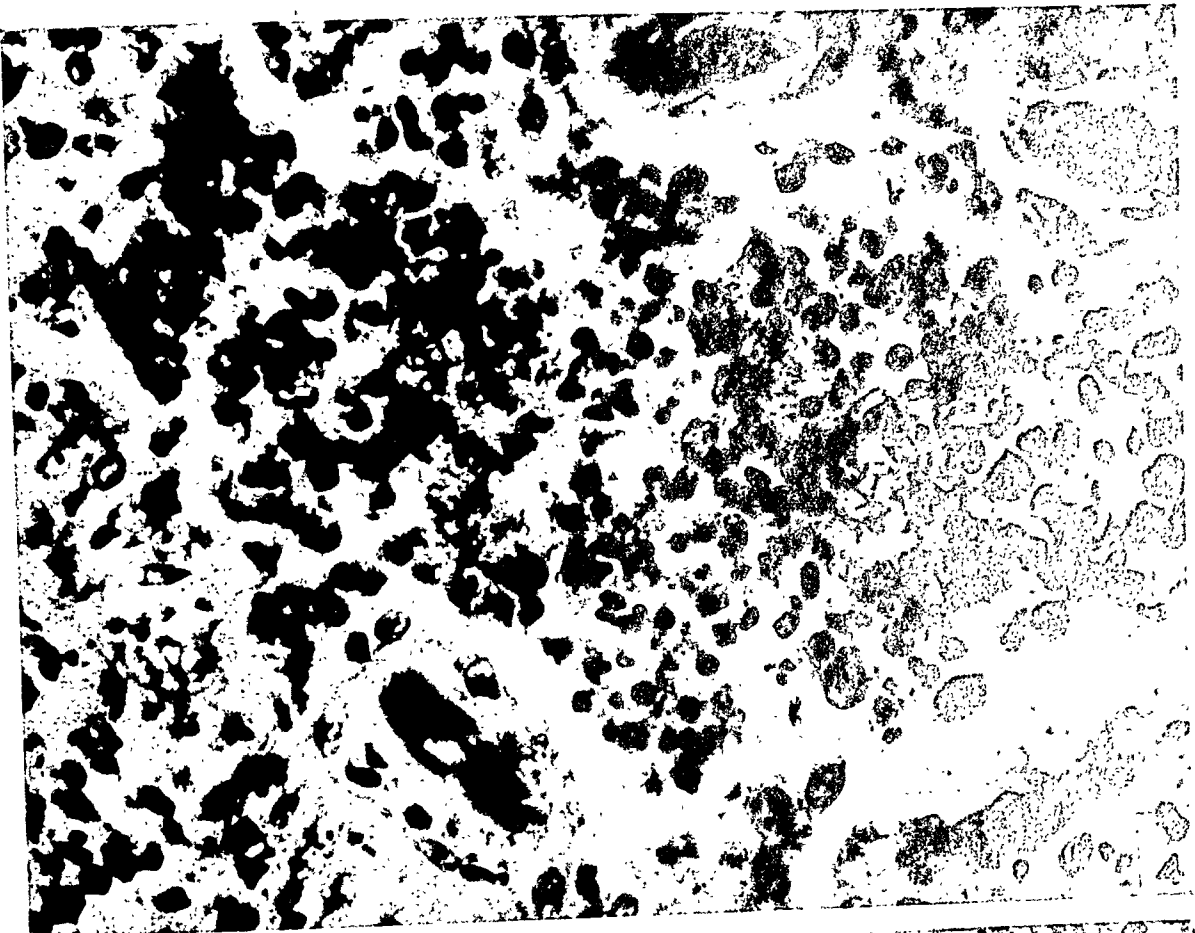


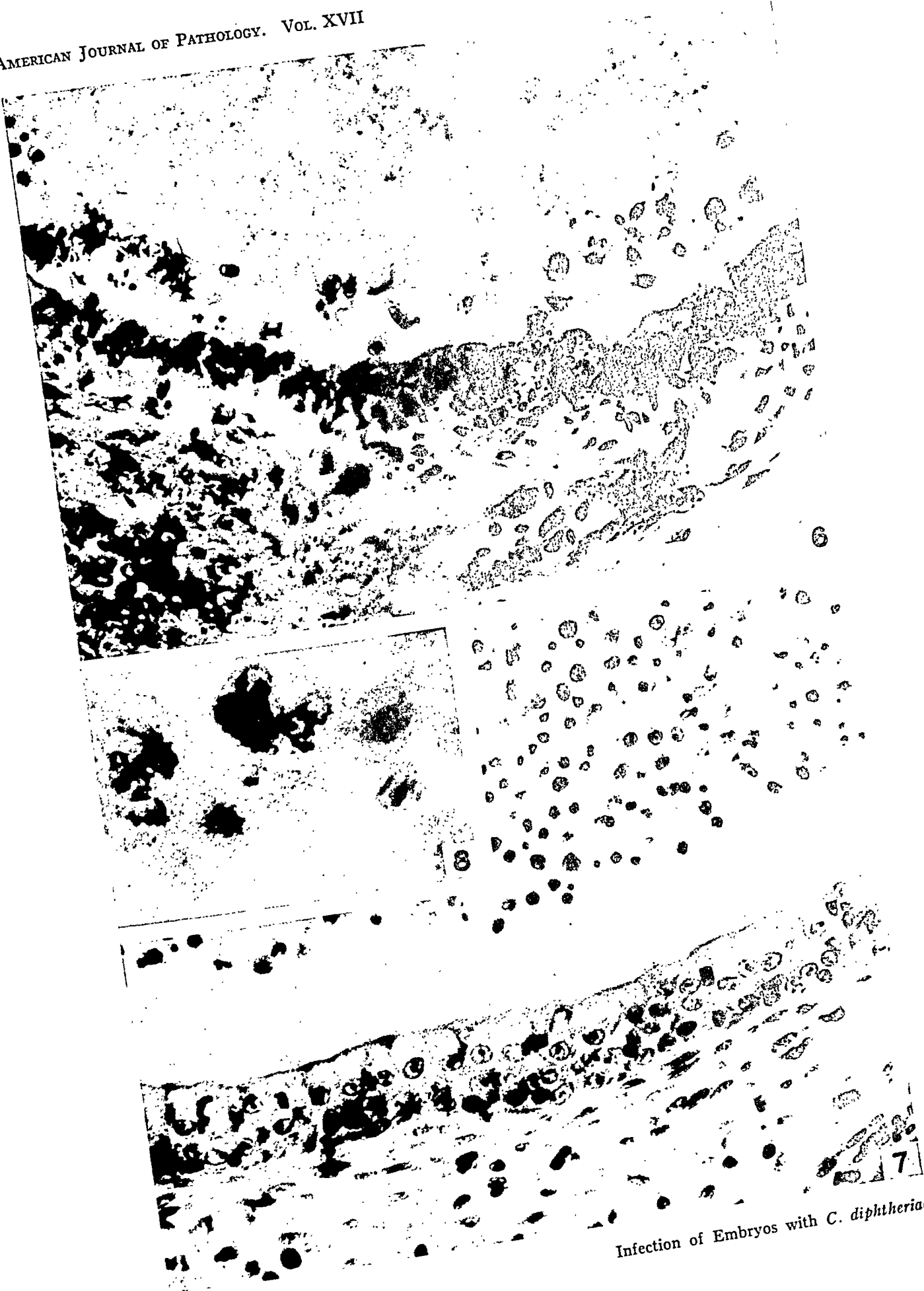
PLATE 82

- FIG. 6. Trachea of unprotected chick embryo showing masses of diphtheria bacilli, a minimal amount of necrotic exudate and necrosis of the mucosa and submucosa. Wright's stain. $\times 500$.
- FIG. 7. Trachea of chick embryo passively immunized with commercial diphtheria antitoxin showing a monocytic exudate in lumen and minimal evidence of necrosis following infection with *Corynebacterium diphtheriae*. Wright's stain. $\times 500$.
- FIG. 8. Higher magnification of exudate shown in Figure 7 showing bacilli in the cytoplasm of large monocytes. Wright's stain. $\times 1900$.



Infection of Embryos with *C. diphtheriae*

Cromartie



Infection of Embryos with *C. diphtheriae*

Cromartie

followed by its disappearance, while at the same time the nucleus became very basophilic, resembling strongly the larger glial nuclei. In the present experimental study on mice I was interested in changes due to rapid starvation and also in those due to more prolonged starvation, in which this nuclear metamorphosis might occur. It seemed probable that a much shorter period of starvation might lead to such changes in mice, if they would occur at all. However, there was no way to verify this but by experiment.

In relation to the glial changes in starvation I wished to see whether the increase in satellitosis and the definite process of neuronophagia would occur in other parts of the nervous system, as in the gray matter of the spinal cord and in the semilunar ganglion. There was further interest in the possibility of definite evidences of neuronophagia in the ventral horn of the spinal cord and in the extent to which such changes might correspond to those reported in poliomyelitis. It was hoped to be able to determine whether the increase in glial cells is due to a migration from other regions or to an actual multiplication of cells in the region involved. Changes in the astrocytes were to be studied by silver carbonate impregnation.

MATERIALS AND METHODS

In the study of the effects of rapid starvation, 8 mice were used. Four of these were pedigreed black male mice, of the strain C57, from the Jackson Memorial Laboratory. Four were white female mice. The black mice were each 110 days old when sacrificed, and the white mice were 20 days old.

For the study of the effects of more prolonged starvation, 6 white mice were used, 5 males and 1 female. These were litter mates, each 65 days old.

In the two groups of 4 animals used in the rapid starvation study, 1 mouse of each group was used as a control, being kept on an adequate and varied diet. The starved animals received all the water needed but no food whatever. Table I shows the degree and rate of weight loss in these animals. They were killed on the fifth day, the starved mice by this time having lost about 25 per cent of their original weight.

The mice used for the study of the effects of more prolonged starvation were weighed every other day and after weighing were

PHAGOCYTIC ACTIVITY OF THE OLIGODENDROGLIA AND AMPHICYTES IN THE BRAIN, SPINAL CORD, AND SEMILUNAR GANGLION OF THE MOUSE DURING INANITION*

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INTRODUCTION

The effects of starvation on the nervous system have been studied by various authors. The changes during rapid starvation, from complete deprivation of food, have been investigated in a number of species of experimental animals. Barrows¹ worked with rats; Urechia and Mihalescu,² with dogs; Garofeanu and Ornstein³ studied the changes in rabbits, and de Couto e Silva⁴ used frogs. Several reports on human material, however, have appeared. Among these are papers by Tarasevich,⁵ Meyers⁶ and Hassin.⁷

In two earlier papers^{8,9} I have reviewed briefly the results of most of these investigators and have reported my own findings. All of the authors who have studied the effects of rapid starvation have described definite nerve cell changes such as loss of Nissl material, shrinkage, vacuolation and disappearance of cells. In my experimental work on the brains of mice,⁸ using controls of the same age as the starved animals, I found, in addition to such changes, a marked grouping of the glial cells about nerve cells in the cerebral cortex, with an active process of neuronophagia or cytolysis of the nerve cells by these "satellite" cells.

In my first report on the subject,⁸ I spoke of the cells active in the process of neuronophagia as being probably microglia, partly due to the classic conception of these cells. Further studies¹⁰ and the employment of the silver technic have led me to the belief that the oligodendroglia are by far the preponderant element in this activity.

In the brain of a man who had wasted greatly due to obstinately refusing food over a period of 9 months I⁹ found, in addition to the cytoplasmic changes of the nerve cells and the glial changes, a peculiar metamorphosis of the nucleus of the neuron. This consisted in a migration of the nucleolus to an eccentric position,

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Immediately after death the brain was removed, together with the cervical and a part of the thoracic portion of the spinal cord. The brain was separated from the cord and cut in half in the sagittal plane. The right half of the brain, together with the cord, was fixed in a 4 per cent aqueous solution of formaldehyde for Nissl staining. The semilunar ganglia on both sides were dissected out and placed in the same solution.

The procedure was the same for all animals except that the left halves of the brains were handled differently in the groups for the study of rapid and of prolonged starvation. Those in the former group were prepared by silver carbonate impregnation, to demonstrate astrocytes and oligodendroglia. Those in the latter group were prepared by Feulgen's method, specific for chromatin, as I was particularly interested in possible nuclear changes in this group. The method followed here was described by Ludford.¹²

The tissues of the 4 black mice used in the rapid starvation experiment were carried through the technical processes, up to embedding, in the same container, with the same fluids, separated by glass partitions with ample room below and at their margins for the free interchange of the reagents. The same procedure was followed for the white mice subjected to rapid starvation and the tissues of the animals suffering a more prolonged starvation, together with their control, likewise were carried through the steps in technic together.

After sectioning and spreading, the sections from the members of each of the three groups were carried through the process of Nissl staining in the same glass slide tray. The sections were stained for 75 minutes in a 1.5 per cent aqueous solution of cresyl violet.

The silver carbonate preparations of the left halves of the brains, designed to demonstrate astrocytes and oligodendroglia, were prepared according to the following method:

1. Fix in formaldehyde-ammonium bromide solution for 24 hours to several days.
2. Cut frozen sections of brain (sagittally, in this case) at 20 to 25 μ .
3. Place in 0.4 per cent aqueous solution of formaldehyde for temporary storage.
4. Wash for 1 minute in distilled water to which 1 drop of strong ammonia for each 5 cc. has been added, to neutralize the formaldehyde.

TABLE I
Rapid Starvation Experiment. Rate and Degree of Loss of Weight

Animals	Weights on succeeding days (grams)				
	1st day	2nd day	3rd day	4th day	5th day
<i>(Black mice)</i>					
B ₁ ♂ (control)	26.7	27.7	27.4	26.8	26.7
B ₂ ♂	31.1	30.3	28.4	27.2	25.5
B ₃ ♂	29.9	27.7	24.9	25.2	21.9
B ₄ ♂	26.5	26.8	23.9	22.6	20.6
<i>(White mice)</i>					
W ₁ ♀ (control)	10.5	10.5	10.9	11.5	12.2
W ₂ ♀	10.7	9.9	8.8	7.8	7.9
W ₃ ♀	10.1	9.5	8.3	7.7	6.8
W ₄ ♀	10.7	9.4	9.3	9.4	8.4

fed a small amount of a commercially prepared laboratory diet. The gradual starvation was continued for 28 days. On the 29th day they were given a final weighing and then sacrificed. Table II shows the average weight for each three successive weighings, made every other day, and the final weight on the 29th day. While there were rather large variations, the loss of weight in general was fairly constant and amounted to between 25 and 30 per cent of the original weight.

The animals were killed by rapid etherization. I had found in earlier work ¹¹ that such etherization had no noticeable effect on the appearance of the nerve cells. Decapitation might have seemed preferable, but after such a process a clean removal of the spinal cord and semilunar ganglia is impossible.

TABLE II
Prolonged Starvation Experiment. Rate and Degree of Loss of Weight

Initial weight		Averages of 3 weighings (Column 1 shows average of 1st 3 weighings, column 2 of 2nd 3 weighings, etc.)					Final weight (Weight at time of killing)
		1	2	3	4	5	
(White mice)							
WP ₁ ♂ (control)	20.0	19.8	19.7	20.3	19.9	22.4	25.8
WP ₂ ♂	22.0	21.1	17.2	15.2	14.5	13.4	13.4
WP ₃ ♂	19.5	16.1	16.1	16.6	17.6	15.4	14.2
WP ₄ ♂	19.5	18.4	14.8	14.6	14.6	13.8	13.4
WP ₅ ♀	20.0	15.8	15.3	14.9	13.7	14.4	14.9
WP ₆ ♂	22.5	20.4	17.1	15.3	15.1	14.7	17.7

cerebral cortex of these starved animals than in those studied earlier, but it was very abundant in the deeper parts of the cerebrum and in the medulla oblongata. Here, as before, I found no action of the glial cells on the Purkinje cells of the cerebellum. These cells, however, did show chromatolysis and shrinkage in the starved animals.

Study of these brains has led me to the conclusion that the increase in numbers of the glial cells is due to amitotic division of these cells in the starved animals. In many instances two nuclei were seen lying in close juxtaposition as though they had just divided, and by more careful searching I found instances of division actually occurring. One of the more striking examples of such division is seen in Figure 2. This division is fairly typical although frequently the nuclei are considerably farther apart at the completion of division than in this picture.

It seems probable that the stage of most active division already was over in these animals, since the rapidly starved animals were already in the last stages of inanition when killed. In the brains of these starved animals I found amitotic division not only in the gray matter but also in the long chains of oligodendroglial cells between the nerve fibers in the white matter.

In the control white mouse (W_1 , rapid starvation) the nerve cells were similar to those in other normal animals of 20 days, most of the cells having large amounts of Nissl material and many having fairly clear, white, vesicular nuclei. In the starved animals, W_2 , W_3 , and W_4 , there were numerous degenerate-appearing nerve cells in the brain with many instances of neuronophagia, again more abundant in the deeper portions of the cerebrum and in the medulla than in the cortex. The increase in glial cells and in the tendency to satellitosis was not noticeably different than in the black mice 110 days old. As in the black animals, there was no neuronophagia of the Purkinje cells, but the loss of Nissl material in these cells in the very young starved animals was striking.

Studies of the glial cells (astrocytes and oligodendroglia) as seen in the silver stain, showed a surprising lack of change on the part of the astrocytes. In all of the starved animals these cells appeared similar to those of normal animals, with long cytoplasmic processes, many of them ending in sucker feet (Fig. 4).

5. Wash in distilled water.
 6. Place section in a 5 per cent sodium carbonate solution for 1 hour.
 7. Wash in distilled water.
 8. Impregnate in silver carbonate solution. This solution was made up as follows:
 - a. Silver nitrate, 30 cc. of a 10 per cent solution.
 - b. Sodium carbonate, 120 cc. of distilled water containing 10 gm. of sodium carbonate.
 - c. Ammonium hydroxide sufficient to dissolve the precipitate.
 - d. Distilled water to make the solution up to 450 cc.
- The impregnation was carried out *in the incubator* at a temperature of 38° C. for a period of 12 to 15 minutes. Heating during impregnation was a feature of the Spanish methods.¹⁸
9. Rinse in distilled water.
 10. Reduce in a 2 per cent aqueous solution of formaldehyde until the sections are a yellowish gray.
 11. Wash in distilled water.
 12. Tone in gold-chloride (1:500).
 13. Fix in 5 per cent solution of photographic sodium hyposulfite for 5 minutes.
 14. Wash in distilled water.
 15. Dehydrate on slide with 95 per cent alcohol followed by carbol-xylol-creosote mixture (1:15).
 16. Mount in balsam.

All sections were examined both with lower powers and with the oil immersion lens.

OBSERVATIONS

RAPID STARVATION

B₁, the control black animal, showed no evidence of degeneration in the cells of the brain, which resembled those of other normal mice of this age, with fairly abundant Nissl material, few satellites, and no neuronophagia. In B₂, B₃, and B₄, the starved animals, the nerve cells in practically all regions of the brain showed degenerative changes, consisting chiefly in chromatolysis and vacuolation. Even more conspicuous, however, was the change in the glial cells, particularly in the oligodendroglial cells. These appeared on low power observation to be increased in number and to have an increased tendency to phagocytosis, clustering about the nerve cells and invading their bodies, apparently by cytolytic action (Fig. 1).

The general picture was very similar to that described in my previous work. There was somewhat less neuronophagia in the

B₂ the nerve cells appeared definitely altered, the chief difference being a diffuse, powdery appearance of the Nissl material. A few of the cells appeared very degenerate, with ragged cytoplasm, and in the neighborhood of such cells there was a definite increase in the amphicytes—interstitial cells with large, oval, vesicular nuclei. The nuclei of the nerve cells did not show any consistent differences between the starved and the fed animals.

In B₃ the Nissl material was definitely less abundant than in B₁ and B₂. There was in addition an irregular vacuolation in perhaps 20 to 30 per cent of the cells. This appeared to be a definitely degenerative change with the smaller vacuoles growing larger, or fusing to form larger vacuoles, until little or none of the normal cytoplasm was left. The amphicytes here were more definitely increased in number and more markedly aggregated about the degenerate cells than they were in B₂. Again, the nuclei did not show consistent differences, although in the more degenerate cells they never presented the clear white, vesicular appearance seen in numerous cells in the normal ganglion, nor were the nucleoli as conspicuously stained in degenerating cells. The Nissl substance was of the diffuse powdery type, apparently not greatly decreased in amount as compared to B₁. The coarse, irregular vacuolation seen in B₃ was as frequently present here. So too were the amphicytes about the degenerate cells. The nuclei were similar to those in B₃.

In W₂, W₃, and W₄, the starved white mice, the Nissl substance was greatly decreased in amount in the majority of the nerve cells. In many cells the large vacuoles of degeneration were present and the cytoplasm was of a ragged appearance, having pulled away at many points from the surrounding capsule. The amphicytes, greatly increased in numbers and gathered about the degenerating cells, could be seen to have invaded the capsules of such cells and apparently were phagocytizing them by a process of cytolysis. While it was impossible to determine in the majority of the cells whether the chromatolysis had been of the centripetal, centrifugal, or of an irregular variety, in a small proportion it was clear that the centripetal variety was occurring, while none of the centrifugal type could be seen. The nuclei of the cells in the semilunar ganglia of the white mice showed no consistent change. In the nondegenerate cells they seemed, if

There were no evidences of clasmatodendrosis (loss of the processes).

The oligodendroglia exhibited a great degree of polymorphism in both normal and starved animals, but in the starved mice they showed also the tendency to divide, to cluster in greater numbers about nerve cells and to hypertrophy, becoming rounder and apparently of a thinner consistency than normally, with cytoplasmic girders connecting the nuclei to the cell wall.

Ventral Horn of the Spinal Cord

Study of the spinal cords of these animals revealed marked differences between the control and starved mice, making the sections from each easily recognizable even with low power observation. In B₁ (control) the large motor cells of the ventral horn contained abundant Nissl material in the form of elongated blocks. The cell outlines were smooth. There were no signs of degeneration, satellitosis was not marked, and there was no neuronophagia. In B₂, B₃, and B₄ the majority of the large motor cells were hypochromatic and vacuolated. There were many satellite cells and conspicuous neuronophagia (Fig. 3). The picture was strikingly different from the normal.

Likewise in W₁ (control) the motor cells were in good shape and even more abundantly supplied with Nissl material than in B₁. There was little satellitosis and no neuronophagia. In W₂, W₃, and W₄ the motor cells were considerably altered. While many of them were hypochromatic, others appeared to have undergone a pyknotic shrinkage, with both nucleus and cytoplasm a deeply basophilic, almost homogeneous mass. The glial cells were increased, satellitosis conspicuous, and many instances of neuronophagia were seen.

Semilunar Ganglion

In the young, amply fed animals, both black and white, the nerve cells of the semilunar ganglia showed smooth outlines, abundant Nissl material in the form of granules or blocks of varying size, and, in the larger cells, frequently clear white, vesicular nuclei, which had conspicuous nucleoli.

The cytological picture of the semilunar ganglia of the starved animals was of a more varied type than in the spinal cord. In

erties of many of the nuclei seen in the sections stained with cresyl violet, it is as yet difficult to say. The subject demands further investigation, and studies along this line are planned.

Ventral Horn of the Spinal Cord

The motor cells of the ventral horn of WP₁ (control) had smooth outlines, abundant Nissl material, and, in most cases, clear nuclei. No evidences of neuronophagia were seen.

In WP₂, WP₃, WP₄, WP₅, and WP₆ the nerve cells presented a definitely altered, although not uniform, appearance. Most of them fell into one of two classes. In the first and more numerous class were found hypochromatic, vacuolated cell bodies and only lightly basophilic nuclei. In the second group, containing about one third of the motor cells, both nucleus and cytoplasm stained very deeply and the processes could be seen extending for considerable distances. The glial nuclei were increased in numbers and there was much satellitosis and definite neuronophagia, which, however, was not so striking as in the more rapidly starved animals.

Semilunar Ganglion

In WP₁ (control) the ganglion cells had abundant Nissl material in discrete blocks or granules, with clear white, vesicular nuclei in the larger cells and lightly basophilic nuclei in the smaller.

In the ganglia of WP₂, WP₃, WP₄, WP₅, and WP₆, there were degenerative changes in the cytoplasm of the cells, consisting chiefly in the replacement of large portions of the normal cytoplasm by coarse vacuoles of varying size, some individual vacuoles occupying over a fifth of the area of the section of the cell. Hypochromatism was a feature of a considerable number of cells, but was not outstanding in an examination of a section of the ganglion as a whole. About many of the more degenerate cells there were aggregations of amphicytes, apparently phagocytizing the nerve cells.

An interesting nuclear change was seen in many of the cells in the animals which had undergone prolonged starvation. The nuclei in perhaps one fourth of the cells were considerably reduced in size, sometimes to less than one half of that which would

anything, to be of a clearer white than in the normal animal, while in the more degenerate cells they appeared to share in the degeneration rather late, by alterations in the nucleolus and warping of the nuclear membrane.

PROLONGED STARVATION

The findings in the brains of these animals will be considered first. In WP₁ (control) the brain presented the normal appearance for an animal of this age (65 days). The cells of the cerebral cortex, the Purkinje cells, and the other cell groups contained abundant Nissl material. The Purkinje cells were of average size, but their nuclei were more uniformly basophilic than those of many young mice which I have examined.

In WP₂, WP₃, WP₄, WP₅, and WP₆ the cells of the cortex and of other areas in the cerebrum were definitely shrunken in appearance, and the nuclei more basophilic than in WP₁. In addition to the increased basophilic properties of the nuclei, in some of the cells (6 to 8 per cent), the nucleoli had become so eccentrically placed within the nuclei as to lie in contact with the nuclear membrane. There appeared to be a definite increase in the number of glial cells (oligodendroglia) and there were numerous instances of neuronophagia, often several in one high-power field. Many instances of two glial nuclei lying in close apposition, as though recently divided, were seen, and examples of amitotic division in progress were found. The Purkinje cells presented an appearance very different from that seen in WP₁. They appeared to be predominantly of two types. One type was that with a small, shrunken, very deeply basophilic cell body and a nucleus so deeply staining that its boundaries were almost indistinguishable. In the other type the cell was often enlarged, somewhat rounded, and always deficient in Nissl material. The entire line of Purkinje cells gave the appearance of having "sunken" back toward the granular layer.

The left halves of the brains of these animals, stained by Feulgen's method for chromatin, showed an increased chromatin content of the nuclei in the cerebral cortex and other parts of the cerebrum, with a less constant increase in the chromatin of the Purkinje cells of the cerebellum. Just how far this increase in chromatin content corresponds with the increased basophilic prop-

had cell bodies fairly intact except at the point of attack by the glial cell or cells.

It is of great interest that Spielmeyer¹⁴ described the phagocytosis of nerve cells in early poliomyelitis (a classic example of "neuronophagia") as carried out by polymorphonuclear cells from the blood vessels, but as of the same nature as the process which he described for the glial cells. He said of this condition: "Auch hier ist es nicht ein primärer Angriff der Zytophagen auf eine gesunde Zelle, sondern eine Einschmelzung der zerfallenden Ganglienzelle durch 'Nekrophagen'." Later lymphocytes and glial cells enter the picture in poliomyelitis. If this is the method of phagocytosis or neuronophagia of the motor cells by these acceptedly phagocytic polymorphonuclear leukocytes of the blood, there is full justification for applying the term "phagocytes" or "neuronophages" to the glial cells which carry on an almost identical process.

The question of what types of cells act as phagocytes in the central nervous system under various conditions is an old one. One of the earliest mentions of the possible or probable rôle of the oligodendroglia, as well as of the microglia in this relation, is found in the report of Robertson¹⁵ on his successful platinum impregnation of the "mesoglia," the glial cells exclusive of the astrocytes. Dr. Clouston, speaking for Robertson in his absence, said of these cells: "Sometimes they have no processes, sometimes two processes . . . The mesoglia cells seem to have a phagocyte action in certain pathological conditions." Most of Robertson's illustrations are typical pictures of the oligodendroglial and some are typical of microglial cells, as seen with the later silver stains.

Penfield¹⁶ denied that the oligodendroglial cells can assume a phagocytic rôle. He would confine such activity to the microglia, which would agree better with their supposedly mesodermal origin, than with the apparently exclusively ectodermal origin of the oligodendroglia. Del Rio-Hortega¹⁷ was inclined to homologize these cells with the cells of the sheath of Schwann and not to assign any phagocytic rôle to them. Cone¹⁸ stated that the oligodendroglia, at least in acute disease of the central nervous system, show only regressive changes, and that this fact supports Penfield's classification of the interstitial cells into neuroglia (astrocytes and oligodendroglia, supposedly of ectodermal origin

be considered normal for a cell of corresponding size, and had taken on a peculiar, glassy appearance, with the nuclear sap a light green in color. The cytoplasm immediately surrounding such a nucleus was usually entirely devoid of Nissl material, while the periphery of the cell was abundantly supplied. Just how much of this appearance was due to an actual centrifugal type of chromatolysis and how much to simple reduction in size of the nucleus, with failure of the Nissl material to move inward to fill in the space thus left, it is difficult to say.

DISCUSSION

The most important conclusions from the present work are those in relation to the nature and extent of the phagocytic processes in the parts of the nervous system here studied.

As a result of rapid starvation, an active process of neuronophagia, carried out predominantly by the oligodendroglial cells, has been found in the cerebrum, medulla oblongata and spinal cord of the mouse. The term neuronophagia is used here to mean a cytolysis and ingestion of the cell bodies of neurons.

Spielmeyer,¹⁴ while he cautioned against confusing "pseudo-neuronophagia" (appearance of phagocytosis in which the satellite cells simply happen to be particularly numerous about a nerve cell or group of cells, or even lie in shallow concavities in such cells) with true neuronophagia, nevertheless recognized a process by which glial cells penetrate and take up the cytoplasm of degenerating ("fluidified") nerve cells. He stated: "Die Gliazellen haben vielfach die Tendenz, in den verflüssigten Zelleib einzudringen und sich an die Stelle der zugrunde gehenden Ganglienzelle zu setzen; wir haben es hier mit einer echten Neuronophagie zu tun." The illustrations of the process in his text show almost undoubted oligodendroglial cells in this rôle.

From observations on this process in great abundance and in all stages both in the brain and spinal cord, I not only can agree with Spielmeyer's statement but can assert that it is the glial cells which in most instances bring about the "fluidification," or lysis, of the nerve cell, probably ingesting it while this process is occurring. Many of the nerve cells observed in process of being phagocytized, both in the brain and in the ventral horn, have

may become transformed into phagocytic cells. Thus Scholz²⁸ stated that in some very chronic conditions the astrocytes perform a function of phagocytosis, probably aided by the oligodendroglia. I have not seen any evidences of such activity, nor indeed of any changes in morphology, in the astrocytes of my animals, either of those starved rapidly or of those starved over a longer period.

The most interesting changes in prolonged starvation, in addition to alterations in the cytoplasm of the nerve cells and the phagocytic activity of the oligodendroglia, are the nuclear changes occurring in many, though by no means all, of the nerve cells. These consist in increased basophilic properties of the nucleus, due probably in part or entirely to an actual increase in chromatin as shown by Feulgen's method. These nuclear changes are shown more or less markedly in the brain, cord, and semilunar ganglia.

SUMMARY AND CONCLUSIONS

The oligodendroglia increase in numbers and carry on a definite phagocytic activity in the cerebrum, medulla oblongata, and spinal cord of the mouse, both in rapid and in prolonged starvation.

In the semilunar ganglion the phagocytic rôle is played by the amphiocytes, cells bearing a resemblance to the oligodendroglia.

I describe this type of process as one of neuronophagia, a cytolytic destruction and ingestion of nerve cells by other kinds of cells, whether by polymorphonuclear cells, as in early poliomyelitis, or by glial cells, as in my own material.

In prolonged starvation there are definite changes in the nuclei of some of the nerve cells, consisting chiefly in increased basophilic properties, probably correlated with the increase in actual chromatin content.

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and never acting as phagocytes) and microglia (supposedly of mesodermal origin and frequently undergoing progressive changes to undertake a phagocytic rôle).

Ferraro and Davidoff¹⁹ believed that they had demonstrated conclusively, by silver impregnation and fat-staining methods, that the oligodendroglia can act as phagocytes and that such cells, as well as the microglia, are transformed into "gitter-cells." They did not, however, bring out the relationship of these cells to nerve cells during this process. Cramer and Alpers²⁰ reported that they had shown a definite phagocytic activity on the part of the oligodendroglia in secondary degeneration in the white matter of the cord, the oligodendroglia passing through a metamorphosis involving acute swelling, among other changes, and playing the predominant rôle in the breakdown and digestion of the degenerate nerve fibers. They compared the phagocytic-digestive activity of the oligodendroglia to that of glandular cells, a comparison which could be followed in my own observations on the digestion of the nerve cell body by these cells. They found in this process also a hyperplasia, or increase in numbers, of the oligodendroglia. Such a hyperplasia has been shown in my material by increase in numbers and by the finding of amitotic division figures.

It is of significance that De Castro²¹ regarded the amphicytes in ganglia as similar to the oligodendroglia. He said of the function of the amphicytes (satellite cells) under pathological conditions: "The digestion or disintegration of the cytoplasm is undoubtedly the work of the satellite cells which perhaps release histolytic ferments elaborated by their cytoplasm." My observations on the semilunar ganglion agree with such a conclusion. I found, also, that oftentimes in these young starved animals the amphicytic nuclei may be spheroid as well as oval, thus increasing their resemblance to oligodendroglia.

Rydberg²² has questioned an exclusively mesodermal origin of the microglia. Studying fetal brains, both of human beings and lower animals, he found both microglia and oligodendroglia having an ectodermal origin. This finding is noted here as showing that the classification of glial cells according to origin can as yet scarcely be taken as a hard and fast one, nor as one on which to base considerations of the probable rôles of the various types. Some few investigators have suggested that even the astrocytes

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DESCRIPTION OF PLATES

PLATE 83

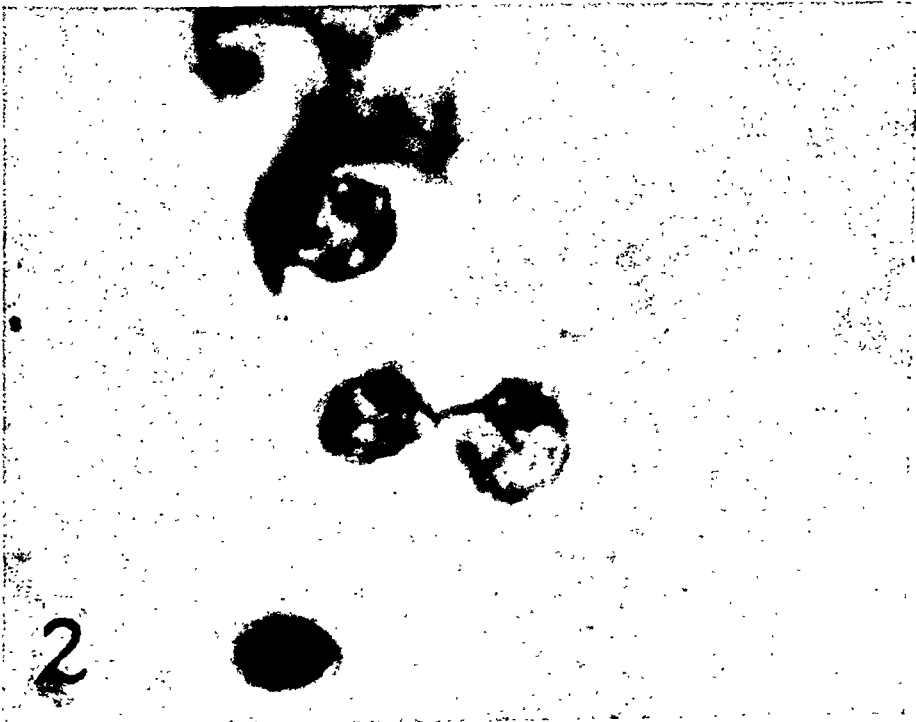
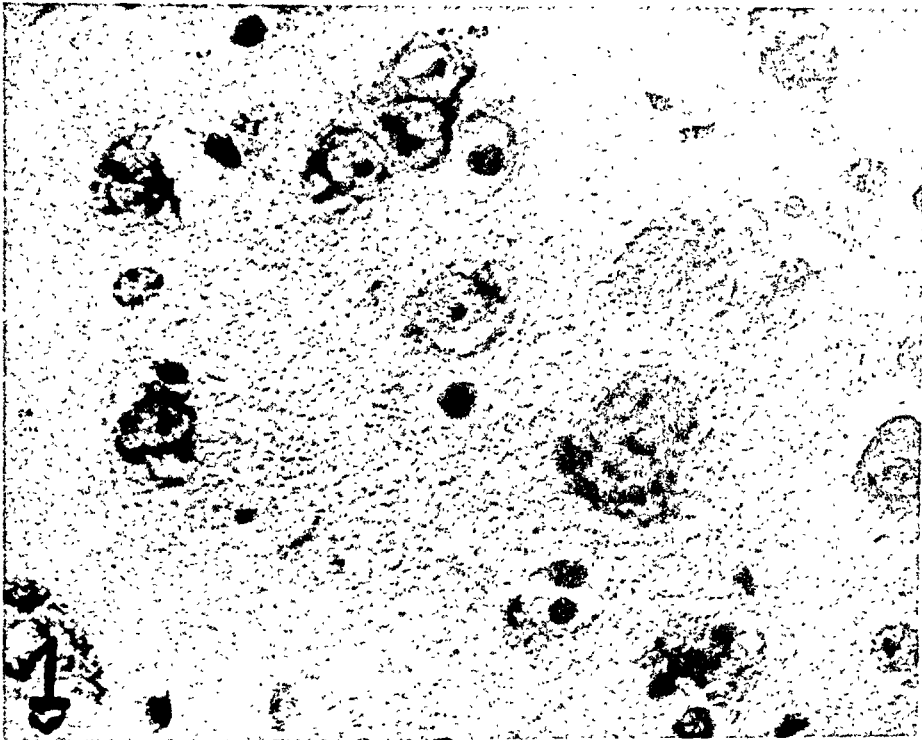
FIG. 1. Cerebrum of a mouse (B₂) starved for 4 days and in the last stages of inanition. The degenerating nerve cells are being invaded and phagocytized by oligodendroglial cells. Nissl stain. $\times 800$.

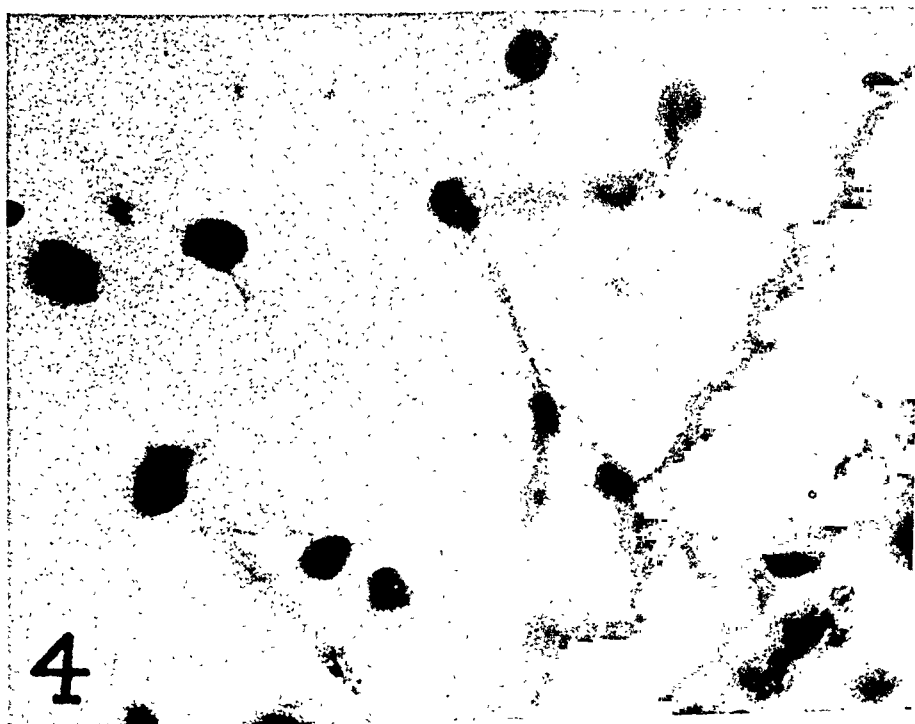
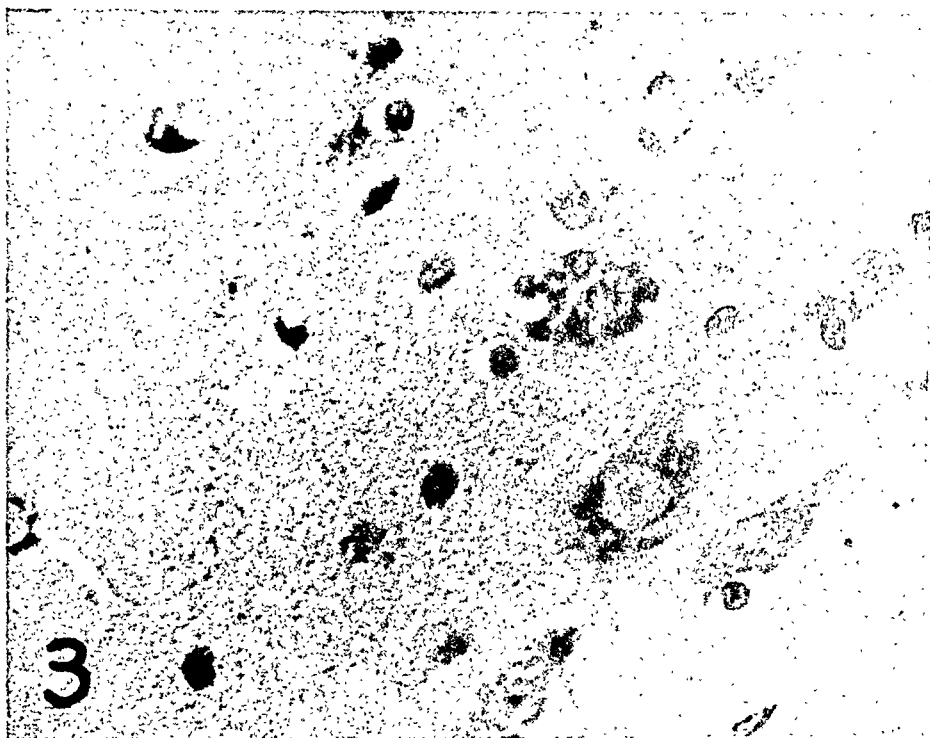
FIG. 2. An oligodendroglial nucleus from the cerebral cortex of the same animal as in Figure 1, caught in the act of amitotic division. The daughter nuclei are not exactly similar here. Above the dividing glial nucleus is another oligodendroglial cell in contact with the crescent-shaped remains of an almost completely phagocytized nerve cell. Nissl stain. $\times 2000$.

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PLATE 84

- FIG. 3. Ventral horn of the spinal cord of a mouse (B_2) starved for 4 days. The motor cells are degenerate and many of them are being phagocytized by the glial cells. Nissl stain. $\times 800$.
- FIG. 4. Astrocytes from the region of Ammon's horn of a mouse (B_3) starved for 4 days. There is no apparent change in these cells. Their processes and sucker feet appear as in normal animals. Silver carbonate method (see text). $\times 800$.





mice the same cellular elements were involved, but minute polymorphonuclear abscesses and giant cells were not noted as in the natural human disease.

The phosphatide of the blastomycete produced in mice much the same tissue reaction as the living organism, but less necrosis, in the same periods of time. Thus the phosphatide appeared not to be responsible for the necrosis associated with the living organism.

The polysaccharide of the blastomycete was not studied to determine whether it had a necrotizing effect, but to see whether it produced the same acute effects in the rabbit as the tuberculo-polysaccharide, as described by Sabin, Joyner and Smithburn in 1938. It did. The blastomycete polysaccharide injected intraperitoneally into rabbits produced mild, sterile, transient, acute peritonitis; and in the blood, leukopenia, lymphopenia, and increase in immature polymorphonuclear cells. Control substances produced less pronounced changes.

FACTORS REGULATING THE ABSORPTION OF IRON IN DOGS AS MEASURED WITH THE RADIOACTIVE ISOTOPE. By W. M. Balfour. *From the University of Rochester, School of Medicine and Dentistry.*

It has been demonstrated that the anemic animal, deficient in iron stores, will absorb considerably larger amounts of iron than will the normal animal. The mechanism of this unusual physiological reaction has been studied from a number of angles. A typical set of experiments follows: A normal animal was shown to absorb 1.3% of a single dose of 130 mg. of iron containing the radioactive isotope. When this animal was rendered acutely anemic by the removal of two-thirds of the circulating blood volume during the course of a few hours, and twenty-four hours later given the same dose of radioactive iron, the amount of absorption was about the same as before, within experimental limits. After being allowed to return to a near normal range of circulating hemoglobin at the expense of the body stores of iron, and again fed radio-iron at the same dosage level, the amount of absorption was 10 per cent instead of less than 2 per cent. This suggests that the level of anemia, *per se*, did not influence the amount of iron absorbed, but that some tissue factor, possibly the mucosal iron of the intestine, was the determining factor.

EFFECT OF GLYCOGEN ON GROWTH OF WALKER 256 TUMOR IN RATS. By Howard A. Ball. *From the San Diego County General Hospital.*

Intravenous injection of commercial practical and chemically pure glycogen solutions cause marked inhibition of tumor growth, while starch, acacia, or glucose, in equal or higher concentrations, produce no effect. Hydrolysis of glycogen abolishes the effect.

NEURAL AND HUMORAL RESPONSES FROM HYPOTHALAMIC STIMULATION IN CAT AND MONKEY. By Morris B. Bender and Edwin A. Weinstein. *From the Laboratories of the Mount Sinai Hospital, New York.*

The hypothalamus was stimulated in cats and monkeys (*Macacca mulatta*) with the Horsley-Clarke technic. Observations were made on the normal pupil and completely denervated iris, and arterial tension. In the *cat* electric stimulation of the dorsal portions of the hypothalamus results in dilatation

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ABSTRACTS OF PAPERS

EXPLANTATION AS AN AID TO THE STUDY OF KIDNEY PATHOLOGY. By Frederick M. Allen and William E. Youland. *From the Departments of Physiology and Pathology, New York Medical College, New York City.*

Explantation of kidneys under the skin was originally introduced by one of the writers with a view to broader purposes than have yet been carried out by authors who have subsequently adopted or claimed the method. This paper gives a preliminary description of some findings by this method, in particular, observations with temporary stasis of circulation and with diet.

THE INFLUENCE OF VARIOUS TYPES OF IMMUNIZATION UPON THE PATHOGENESIS OF EXPERIMENTAL ARTHRITIS. By D. Murray Angevine. *From the Department of Pathology, Cornell University Medical College, New York City.*

In the first part of the study, 107 rabbits were immunized, and 99 served as controls. The animals were immunized with either repeated intradermal or intravenous injections of heat-killed hemolytic streptococci over a period of 8 weeks. They were then infected with intravenous injections of homologous cultures. Histological studies were made on the joints, heart and kidneys in every instance. These experiments indicate that arthritis was produced more frequently and with smaller doses of bacteria in the intravenously immunized animals than in either the intradermally immunized or control rabbits.

The second part of the experiment, carried out on 81 rabbits, deals with the effect of intra-articular injection of heat-killed streptococci or nucleoprotein into one knee joint, and heat-killed staphylococci or horse serum into the contralateral joint. The animals were then infected intravenously with hemolytic streptococcus cultures and killed at various intervals. In agreement with the earlier work of Swift and Boots, no conspicuous differences were noticed in the clinical arthritis; however, cultural and histological studies were made on the synovial membranes of all the joints. It was found that the hemolytic streptococci were recovered with more difficulty from the intra-articularly injected joints, and although the clinical and gross findings were not conspicuous, the histological changes are of considerable interest.

TISSUE REACTIONS TO *Blastomyces dermatitidis* AND TO ITS PHOSPHATIDE AND POLYSACCHARIDE FRACTIONS IN ANIMALS. By Roger D. Baker. *From the Department of Pathology, Duke University Medical School, Durham, N.C.*

The tissue reaction in experimental blastomycosis of 2 to 6 weeks duration in mice differed from that noted in spontaneous human blastomycosis. In

the choline-containing phospholipids (lecithin and sphingomyelin) and a slight increase in the cephalin of the blood. The phospholipid and cholesterol of the blood of rabbits receiving phospholipid in addition to the cholesterol added to the diet was similar to those receiving cholesterol alone. In rabbits which received phospholipid without added cholesterol, the blood phospholipid was elevated about 25% above the control values.

The rabbits receiving cholesterol developed marked atherosclerosis in from twenty to forty weeks. Microscopic lesions, similar to the early atherosclerotic changes, appeared in the aortas of rabbits fed phospholipids for 30 to 40 weeks although the blood cholesterol of these animals was not greatly elevated at any time during their course. These studies confirm the well established facts concerning cholesterol arteriosclerosis, and further suggest that the elevation of the phospholipids of the blood is a factor which merits further consideration.

DIFFUSION OF SOME ANIMAL VIRUSES. By Jaques Bourdillon. *From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia.*

The author's analytical diffusion method has been applied to the study of viruses of influenza A, vaccinia, and mouse encephalomyelitis.

Diffusion of influenza (in mouse lung suspension). The results showed that the diffusing virus was not homogeneous, but consisted of two fairly distinct fractions: one fraction, representing about 99% of the total virus, diffused slowly and appeared to consist of particles with a diameter of the order of 200 millimicrons; the other fraction, representing about 1% of the total virus, diffused fast, and appeared to have a particle diameter of the order of 6 millimicrons.

Diffusion of vaccinia (from rabbit skin). The results were similar to those obtained with influenza. They suggested that most of the infectivity was present in particles of the order of 200 millimicrons in diameter, but that about 0.1 or 0.01% of the virus was present in particles about 6 millimicrons in diameter.

Diffusion of mouse encephalomyelitis (from mouse brains). Diffusion showed the material to be very inhomogeneous. About 10% of the virus present appeared to have a particle diameter of about 15 millimicrons; smaller particles may have been present.

The tentative conclusion has been drawn that the elementary bodies or particles currently described as the physical units of animal viruses may be only virus carriers, and that the actual virus units are of the size of low molecular weight proteins.

FURTHER OBSERVATIONS ON A GASTRIC SECRETORY DEPRESSANT FACTOR IN GASTRIC JUICE. By Alexander Brunschwig and Richard A. Rasmussen. *From the Department of Surgery, University of Chicago.*

Previous studies showed that achlorhydric gastric juices of patients with a gastric cancer, and of patients with pernicious anemia when injected intravenously into dogs with large gastric pouches produced a transitory reduction in secretion volume, and an achlorhydria in over 70 per cent of samples tested. The dose employed was 1 cc. of juice per kilo weight of the dog.

of the normal pupil. This is due to parasympathetic inhibition since oculomotor nerve section abolishes this mydriasis. Stimulation of the ventral portions of the hypothalamus yields both neural and humoral "sympathetic" effects. The neural responses are: (a) prompt maximal dilatation of the normal pupil which is not abolished by section of the oculomotor nerve but is greatly reduced after interruption of the cervical sympathetic trunk, (b) retraction of the nictitating membrane, and (c) prompt increase in the blood pressure. None of these responses is abolished by adrenalectomy. The *humoral* responses are: (a) delayed mydriasis in the completely denervated iris occurring 9-20 seconds after the application and persisting after cessation of the stimulus, (b) delayed fall in blood pressure which is synchronous with the appearance of the mydriasis in the denervated iris. This is frequently preceded by the prompt (neural) rise in blood pressure. Bilateral adrenalectomy abolishes the delayed mydriasis and depressor effects. Intravenous injection of adrenalin, 0.001 gamma per kilo per second, reproduces the "humoral" effect obtained with hypothalamic stimulation.

In the *monkey*, the neural mechanism of pupillary dilatation is predominately that of sympathetic excitation. Stimulation in most hypothalamic areas produces a prompt mydriasis which is greatly reduced or abolished by section of the cervical sympathetic trunk. Parasympathetic inhibition is difficult to detect. The arterial tension is usually raised but never as high as in the cat. In marked contrast to the cat, conspicuous humoral effects are never seen; infrequently a slight delayed "humoral" mydriasis may be observed only after an intramuscular injection of cocaine.

THE EFFECT OF ATROPINE AND PILOCARPINE UPON THE SPHINCTER OF ODDI IN HUMAN SUBJECTS. By George S. Bergh. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The effect of atropine and pilocarpine upon the sphincter of Oddi was studied in patients who previously had undergone cholecystectomy and choledochostomy. The resistance to flow of fluid from the bile duct into the duodenum was measured as "sphincter resistance."

In eleven experiments atropine sulphate was administered subcutaneously in dose of one-fiftieth to one one-hundredth of a grain. In ten cases there was no significant change in the intramural resistance, and in one case the resistance was slightly decreased.

In eight experiments the effect of the subcutaneous injection of six milligrams of pilocarpine was studied. In seven patients there was no change in the sphincter resistance, and in one case relaxation occurred.

These findings suggest that the parasympathetic nervous system does not play an important rôle in the regulation of sphincter activity in human subjects.

BLOOD PHOSPHOLIPID CHANGES IN EXPERIMENTAL CHOLESTEROL ARTERIO-SCLEROSIS. By Jesse L. Bollman and Eunice V. Flock. *From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.*

Rabbits fed cholesterol for several weeks develop a tenfold increase in the cholesterol of the blood. The total phospholipid content of the blood increases to twice its normal value. Most of this increase is due to increases in

STUDIES ON INSULIN-ALLERGY. By Paul R. Cannon and Charles E. Marshall.
From the Department of Pathology, University of Chicago.

Experiments are described concerning the antigenic properties of insulin. Evidence is presented that, in patients proved by skin-tests to be allergic to insulin, the serum contains a specific antibody, demonstrable by the collodion particle agglutination method and by the Prausnitz-Küstner reaction. Discussion is directed to the possible relationship of this antibody to some instances of insulin-resistance.

A STUDY OF THE RELATIONSHIP BETWEEN THE VIRUS OF INFLUENZA A AND FILTERABLE COMPONENTS OF NORMAL LUNGS. By Leslie A. Chambers and Werner Henle. *From the Childrens Hospital of Philadelphia, and the Johnson Foundation, University of Pennsylvania, Philadelphia.*

Particles sedimented from Berkefeld filtrates of emulsified influenzal mouse lung by moderately high speed centrifugation possess the major part of all the infectivity of the original filtrate even after repeated washing. Normal mouse lungs yield quantitatively similar sediments. The normal and infectious lung sediments have been studied simultaneously by detailed chemical analysis, dark field and electron microscopy, specific staining, pycnometry, and spectroscopy. No significant differences between the normal and pathogenic particles have been found, excepting the infectious property.

Such evidence indicates that a component of normal lungs either acquires infectivity by some unknown structural rearrangement, or that it adsorbs an infectious unit of much smaller size than that indicated by ultrafiltration studies. Extremely high infectious titres in a small percentage of the purified preparations, and failure to sediment completely the infectivity of some mouse lung filtrates point toward the latter view. Furthermore, antiserum against particles derived from normal mouse lungs agglutinates extensively the infected preparations and the major part of infectivity is to be found in the agglutinate. This suggests a carrier function of the normal cell constituent.

As the result of a search for a source of virus relatively free from cellular elements, it was found that the extra-embryonic fluid harvested from infected chick embryos is usually fatal for mice in high dilutions. A large part of the infectivity fails to sediment under conditions which remove it almost completely from mouse lung filtrates. Sedimentation, and diffusion data indicate an infectious unit of relatively small size, probably of the order of 10 millimicrons in diameter.

Adsorption with normal lung particles removes most of the infectivity from the extra-embryonic fluid, and transfers it to the particles. This strongly supports the concept of a carrier rôle for the normal cell particles.

RELATION OF AGE TO THE SUSCEPTIBILITY OF THE ERYTHROCYTE TO HYPOTONIC SALINE SOLUTION. By W. O. Cruz. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

Radioactive iron when administered to an animal in which there is a stimulus for hemoglobin formation is promptly converted to new hemoglobin and remains in the red cell until that cell undergoes disintegration.

As controls, acid gastric juices, neutralized before injection, obtained from patients not exhibiting the above diseases, were employed and gastric secretory inhibition was observed in approximately 20 per cent of the samples.

The secretory depressant factor is present in the white flocculent precipitate obtained when four volumes of absolute alcohol are added to the juice. The incidence of samples among the controls exhibiting the gastric secretory depressant was thought to be too great to be regarded as "chance." In order to test the hypothesis that the depressant found in the juice might be evidence of a normal chalone mechanism, hyperactive secretion of the stomach and achlorhydria, and in pernicious anemia, much larger quantities of control samples of juices were injected into the controls, the results of which will be reported.

THE FUNCTIONAL ACTIVITY OF SMOOTH MUSCLE TUMORS OF THE UTERUS

By Charles S. Bryan and Shields Warren. *From the Laboratory of Pathology, New England Deaconess Hospital, Boston.*

No information has been available as to whether or not the smooth muscle cells of a leiomyoma can contract. The following study was carried out on strips taken from leiomyomas of the uterus and from the normal smooth muscle of the wall of the same uterus as controls.

The strips were excised promptly after operative removal of the specimen and immersed in oxygenated Ringer's solution kept at 38° centigrade, and the muscle strips attached by threads to a pointer on the kymograph. The lower ends of the strips were anchored. Contractions were obtained with pituitrin ($\frac{1}{4}$ cc. in 250 cc. of Ringer's solution) from 7 leiomyomas, and with histamine (0.5 cc. of 1/1000 in 250 cc. of Ringer's solution) from 6 leiomyomas. Some strips failed to contract, and showed either much fibrosis, or muscle cells not in the long axis of the strip.

OBSERVATIONS ON THE INFECTION OF CHICK EMBRYOS WITH *B. tularensis*, *Brucella* AND *P. pestis*. By G. John Buddingh and Frank C. Womack. *From the Department of Pathology, Vanderbilt University School of Medicine, Nashville.*

Developing chick embryos are susceptible to infection with *B. tularensis*, *Brucella abortus*, *Brucella melitensis* and *Pasteurella pestis* by chorio-allantoic inoculation. Each of these infections induces characteristic reactions in the chorio-allantois and the embryo proper. Notable differences appear in the early stages of infection, especially in relation to the behavior of these micro-organisms in respect to various intracellular environments. In the infected chorio-allantois *B. tularensis* exhibits a marked predilection for growth within the epithelial cells of the ectoderm. The *Brucella* strains show a much greater affinity for endothelial cells than for ectodermal epithelium. *Pasteurella pestis* grows readily within ectodermal epithelium and in the intercellular spaces. Generalization of these infections to the embryo proper induce characteristic and distinct lesions. Important features in the behavior of these micro-organisms in relation to the early stages of the pathogenesis of infection are emphasized by these observations.

INFLUENCE OF DIET ON LIVER LESIONS CAUSED BY EXCESS DIETARY CYSTINE.

By David P. Earle, Jr. and Joseph Victor. *From the Research Service, First Division, Welfare Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.*

Albino rats fed a low fat (5 per cent) diet with 10 per cent cystine showed portal necrosis and hemorrhage after 2-3 days and portal cirrhosis of the liver as early as 5 days. Fatty infiltration of the liver occurred in rats surviving 6 or more days. These lesions were uninfluenced when this low fat diet was low in protein (5 per cent), high in protein (40 per cent) or high in yeast (20 per cent). High fat (25 per cent), low protein (5 per cent) with 10 per cent cystine produced longer survival and delayed the appearance of cirrhosis to 2 weeks but did not affect necrosis and hemorrhage. Fatty infiltration was greater than that of the preceding groups. Rats fed the McCollum stock diet with 10 per cent cystine showed less fatty infiltration and the incidence and severity of the acute and chronic lesions were diminished.

High fat (25 per cent), low protein (5 per cent) diet with 5 per cent cystine resulted in liver lesions resembling those following 10 per cent cystine but their incidence and severity was diminished. However, fatty infiltration was more severe. One per cent choline added to this diet depressed the fatty infiltration but increased the incidence and severity of the cirrhosis. The McCollum diet with 5 per cent cystine had the same effect as the 1 per cent choline in the aforementioned diet.

THE NON-NEOPLASTIC HEPATIC CHANGES IN RATS FED DIMETHYLAMINOAZOBENZENE WITH SPECIAL REFERENCE TO PIGMENT DEPOSITS. By Jesse E. Edwards and Julius White. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

More than 200 rats were fed dimethylaminoazobenzene (butter yellow) continuously and autopsied at varying intervals from the start of the experiment. Among the changes in the liver exclusive of tumor formation there is cirrhosis with the presence of at least two pigments, one containing iron and one free of iron. The latter pigment is insoluble in the usual organic solvents and is apparently of lipoidal nature.

The cirrhosis is associated with varying degrees of proliferation of bile ducts to the point of grossly visible areas containing no parenchymal cells and only numerous tortuous biliary channels. Cysts of varying size and number are present. These appear to be dilated bile ducts.

The demonstration included lantern slides showing the tinctorial reactions of the pigments and the appearance of the bile ducts and cysts.

EFFECTS OF HYPOPROTEINEMIA ON BLOOD PRESSURE IN NORMAL AND HYPERTENSIVE DOGS. By Cyrus C. Erickson. *From the Department of Pathology, Duke University Medical School, Durham, N.C.*

The following observations show that depletion of circulating plasma proteins in hypertensive dogs is associated with a depression of established blood pressure levels, and to a lesser degree a response of like nature is noted in normal dogs.

Normal and hypertensive dogs have been studied during periods of hypo-

If the isotope is given in a single dose, the age of the red cells incorporating the radioactive hemoglobin can be determined. The physical and chemical properties of these labelled cells can then be compared with the same properties of the older red cells in the circulation.

It can be shown that the *newly-formed red cells* containing the radio-iron are much more susceptible to treatment with hypotonic saline than the other circulating cells of the dog. This susceptibility of red cells to hypotonic saline solution decreases progressively until at about three days of age the circulating red cells are all equally sensitive to hypotonic solution.

BACTERIOGENIC HEMAGGLUTINATION. By I. Davidsohn and B. Toharsky.
From the Department of Pathology, Mount Sinai Hospital, Chicago.

It was previously reported that a gram-positive bacterium designated as *Corynebacterium H* was isolated, which produced panagglutinating properties in serum and plasma and panagglutinability of red cells. Various features of the newly developed antibody-like bacteriogenic property of the serum were described.

The present paper shows:

1. The pH of inoculated serums was found slightly lower than the pH of the uninoculated controls, but the change was not related to the development of bacteriogenic hemagglutinins.
2. Concentrations of certain bacteriostatic agents were established capable of inhibiting bacteriogenic transformation.
3. Yeast extract and certain components of the vitamin B complex favored the development of hemagglutinating properties in serums.
4. The component of the serum which is essential for the transformation by the bacterium was destroyed by a temperature of 65 C. The fraction of the serum which is needed for the development of the bacteriogenic agglutinins is used up in the process of transformation. After removal of the agglutinins by proper adsorption no further transformation occurred.

THE PHYSIOLOGICAL AND PHARMACOLOGICAL BEHAVIOR OF THE HUMAN APPENDIX. By Clarence Dennis. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Previous work from this laboratory has indicated that experimental obstruction to the lumen is capable of producing acute appendicitis in man, the chimpanzee and the rabbit.

The muscular activity of the human appendix has been studied both in freshly excised specimens and in appendixes made available by appendicostomy.

Although the muscular activity of the appendix is somewhat less than that of the ileum, it was demonstrable in 29 out of 32 uninflamed excised specimens, less often in inflamed ones. The effects of a variety of drugs were found in general similar to those in the ileum.

Sufficient muscular activity is demonstrable to suggest that this may play a rôle in initiating appendicitis through luminal obstruction.

determination of the pancreatic enzymes in the duodenal contents permits, on the basis of present evidence, the only positive differentiation between the relatively benign celiac disease, for which there is adequate medical treatment, and the more serious condition, pancreatic fibrosis.

PATHOGENESIS OF INTESTINAL RADIATION LESIONS. By Nathan B. Friedman and Shields Warren. *From the Department of Pathology, Harvard Medical School, Boston.*

Studies have been made of the development of intestinal lesions following radiation of the abdomen. Special attention was given to the pathogenesis of ulcers, and the rôle of various factors in the development of these ulcers was tested experimentally. This was done by means of surgical procedures directed at altering the normal conditions existing in a given loop of bowel in the intact animal. Interference with blood supply, immobilization by adhesions, obstruction by stenosing ligations, and deviation of the fecal stream by colostomy were carried out.

EFFECTIVE RENAL BLOOD FLOW, FILTRATION RATE AND FUNCTIONAL EXCRETORY MASS IN ESSENTIAL HYPERTENSION; DIODRAST AND INSULIN CLEARANCES. By Piero P. Foa, Ward W. Woods and Max M. Peet. *From the Department of Surgery, University of Michigan, Ann Arbor.*

Interest in the relation of renal blood flow and hypertension has been greatly stimulated by the work of Goldblatt, Page and Smith and their respective associates.

Eighteen patients with essential hypertension have been studied with respect to renal circulation. The renal blood flow was reduced in all cases, ranging as low as 145 cc. of blood per minute, as compared with the average normal blood flow of approximately 1200 cc. per minute. The filtration rate was greater than normal, indicating increased glomerular pressure. This may be a consequence of a) the increased systemic pressure, or b) a hypertonus of the efferent glomerular arterioles. Functional excretory mass (diodrast Tm) is below the normal range. The ratio between blood flow and diodrast Tm is also reduced, indicating a relative ischemia of the functioning tissue.

The patients are being studied approximately two weeks and six months after supradiaphragmatic splanchnicectomy also.

In every patient we determine the ratio of the thickness of the wall to the diameter of the lumen of the arterioles of the skeletal muscle in biopsied specimens. This will enable us to correlate the renal circulation and function with the status of the arterioles in the skeletal muscle and the appearance of the vessels of the eyegrounds.

HISTOLOGIC CHANGES IN THE SKIN OF MICE FOLLOWING RADIATION FROM MERCURY ARC. By Hugh G. Grady, Harold F. Blum, and John S. Kirby-Smith. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Male mice of strain A were subjected to radiation from an intermediate pressure mercury arc in quartz. The dosage was accurately measured for wave lengths 3130 Å and shorter by means of a titanium photocell. The in-

proteinemia with daily observations of blood pressure levels. Hypertension was produced experimentally by the Goldblatt renal artery constriction method. Hypoproteinemia was induced by intravenous injections of 6 per cent gum acacia in Locke's solution. The plasmapheresis method was also used as a control method to obviate the known reduction of red cell volume, lowering of cholesterol, and liver injury which occurs with acacia administration.

Associated with hypoproteinemia a definite depression of blood pressure levels occurred in both hypertensive and normal dogs; the more conspicuous drop is demonstrated in the hypertensive dog. The total plasma protein level, the jugular hematocrit, and the fibrinogen show the characteristic drop in the acacia experiments described by several investigators. Plasma depletion experiments tend to indicate that hematocrit variations are not of significance in the depressor effect of hypoproteinemia associated with the acacia injections.

These observations suggest that the acacia administration has no specific pressor effect on the blood pressure, but that plasma protein depletion is probably the significant factor in the recorded blood pressure variations. It is our belief that we have quantitatively reduced specific blood protein or protein-like substances which are necessary components of the humoral mechanism maintaining a "pressor-depressor balance." These observations are compatible with recent experimental evidence of "chemical mediators" removed from the blood of hypertensive dogs.

THE RELATION OF THE PANCREAS TO THE CELIAC SYNDROME. By Sidney Farber and Charlotte Maddock. *From the Department of Pathology, Harvard Medical School, Boston.*

In the pancreases of more than 40 patients suffering from one variant of the celiac syndrome, called for convenience "pancreatic fibrosis," there has been found with regularity a series of changes beginning with an alteration in the character of the secretions in the acini (Wolbach) and passing through stages which include dilatation of ducts, atrophy of acinar structures and eventually marked fibrosis of the gland. Important interference with the formation and liberation of pancreatic enzymes was suggested by these findings. Accordingly, approximately 50 determinations have been made, in a preliminary survey, of the pancreatic enzymes (trypsin, amylase, lipase) in the duodenal contents of three types of patients: (1) normal infants and children, (2) patients suffering from the pancreatic fibrosis variant of the celiac syndrome, and (3) patients suffering from celiac disease. Pancreatic enzymes were greatly reduced or absent in the duodenal contents of four patients in whose pancreases histologic changes were found at autopsy (pancreas fibrosis group). In one of these the alterations were confined to the acini; no obstruction was present in the larger ducts. In the group classified clinically as celiac disease, none of whom has died, the pancreatic enzymes were all within normal range. These studies indicate that (1) interference with the formation or liberation of pancreatic enzymes plays an important rôle in the pathogenesis of pancreatic fibrosis variant of the celiac syndrome; (2) the pancreatic enzymes may be abnormally low at a time before gross obstruction to the ducts has occurred and when alterations only in the acini may be demonstrated on histologic examination; (3) the

can also be demonstrated that in the normal dog the greater fraction of the red cells of the body are in active circulation at all times, since the circulating volume and total volume of red cells as estimated by this direct method are nearly the same under the conditions studied.

STUDIES OF CARTILAGE: I. SOME EFFECTS OF MEDIA pH 4-12 ON THE COMPOSITION OF CARTILAGE. By George Hass and B. Garthwaite. *From the Department of Pathology, Cornell University Medical College, New York City.*

Fresh frozen sections of infant epiphyseal cartilage in controlled lots of 50-80 mg. were extracted with buffer media, pH 4-12, and with dilute and concentrated salt solutions at neutrality for 24 hours at 5° C. Loss of weight incidental to extraction was determined and acid hydrolysates of 5-6 mg. samples of the extracted tissues were quantitatively analyzed for sulphate and reducing substances.

It was concluded that chondroitin-sulfuric acid comprises about 20 per cent of dried cartilage after extraction with 1 per cent aqueous NaCl. A small fraction of this quantity was extracted with 10 per cent aqueous CaCl₂ and a large fraction was extracted at pH 11, while complete extraction in the range pH 4-12 was possible only at pH 12. The complete removal of the polysaccharide in the range pH 7-12 did not account for an additional extraction of 10 per cent of the total weight of the sample, 20 per cent of the total reducing substances and 20 per cent of the total sulphate. Hence, after complete polysaccharide extraction, each section of cartilage possessed 70 per cent of the neutral extraction weight, 30 per cent of the maximum quantity of derivable reducing substances and no sulphate. The sections retained such form after all extractions that they were suitable for correlative morphological studies.

THE PRODUCTION OF GASTRIC AND DUODENAL ULCER IN VARIOUS ANIMALS BY THE INTRAMUSCULAR IMPLANTATION OF HISTAMINE IN BEESWAX. By Lyle J. Hay, David Lynn, and Owen H. Wangenstein. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The experimental production of ulcer in cats by the implantation of histamine in beeswax was described previously from this laboratory by Walpole, Varco, Code and Wangenstein. Subsequently, chronic ulcers were produced similarly in the dog (Varco, Code and Wangenstein). Extension of the method to other animals has shown that chronic and perforated ulcers can be produced in a number of animals by this means. The various species' differences noted will be related. These and previously reported findings emphasize the importance of acid in the genesis of ulcer.

By this method we were able to obtain chronic peptic ulcers in the swine, guinea pig and chicken, and numerous superficial ulcerations, erosions and submucosal hemorrhages in the woodchuck, monkey, rabbit and calf.

Colored photographs and photomicrographs of the specimens were projected. Likewise, typical gastric acidity curves following single injections of histamine were shown.

tensity was 4×10^4 ergs per second per cm^2 and a total dosage of approximately 10^8 ergs per cm^2 per day was given, as determined by the photocell.

Three groups of mice were exposed to this intensity for one, two and five days respectively. The histologic changes in the skin of the ears were essentially similar and were characterized by an inflammatory reaction of slow onset and evolution as well as by rapid proliferation of the epidermis. Gross erythema and microscopic evidence of an inflammatory response were not observed for 2 days following initial exposure. Hyperplasia of the epidermis progressed rapidly and well differentiated prickle cell and granular layers were formed. In 10 weeks following irradiation the epidermal changes had almost completely regressed although there was residual fibrosis of the underlying tissues.

In two other groups of similar mice radiation with 38 per cent and 23 per cent respectively, of the above intensity and dosage was continued 5 days per week throughout the course of the experiment. The early reactions of the epidermis and subjacent tissues closely paralleled those observed in the first three groups. However, at the end of 10 weeks there was no regression of epidermal hyperplasia and the inflammatory reaction was still active.

METHOD FOR TITRATING VIRULENCE OF TUBERCLE BACILLI IN MICE. By F. D. Gunn and John Sheehy. *From the Department of Pathology, Northwestern University School of Medicine, Chicago.*

Cultures of tubercle bacilli are emulsified in saline and standardized by a method previously described. Graded doses are injected intravenously into mice of an inbred strain. Death occurs in three to four weeks from pulmonary tuberculosis after the injection of large doses (0.1 to 1.0 mg.) when highly virulent strains such as the Ravenel strain are used and in five to six months with doses of 0.00001 mg. With strains of low virulence, large doses are required to produce death in mice. The results are much more consistent than those with the usual guinea pig technique.

RED BLOOD CELL VOLUME, TOTAL AND CIRCULATING, AS DETERMINED WITH RADIOACTIVE IRON. By P. F. Hahn. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

By repeated intravenous administration of radioactive iron to an iron-depleted anemic dog, it is possible to raise the level of erythrocytes containing the isotope to a point where the cells of this animal may be used to study the volume of red cells in other dogs.

Cells from this donor animal, containing a known amount of radioactivity, are injected into the vein of an animal under study. Blood samples are taken at various intervals thereafter, and the dilution of the radioactive erythrocytes is determined. It is thus possible to study the circulation time of the red cells and also the circulating volume of these cells. Since there is no loss of the isotope from the cells until decomposition of the cells takes place, a blood sample taken several days after the injection allows the determination of the total red cell volume as well. Plasma volume is determined simultaneously with the red cell volume.

It is found that the determination of the circulating volume of erythrocytes by the dye dilution method may be in error by 20 to 30 per cent. It

the glucose content of the blood in the right side of the heart differed from that in the left side. The duration of the postmortem interval, the temperature of the postmortem environment, the manner of death and the functional state of the liver and pancreas immediately prior to death were important factors in determining the rate and amount of the postmortem change.

ACUTE NECROTIZING ARTERITIS, AORTITIS, AND AURICULITIS FOLLOWING URANIUM NITRATE INJURY IN DOGS WITH ALTERED PLASMA PROTEINS.

By Russell L. Holman. *From the Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, N.C.*

During experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level, it was observed that 8 out of 10 dogs (80 per cent) subjected to alterations in their plasma proteins by plasmapheresis or plasma injections, then given uranium nitrate subcutaneously, showed well marked necrotizing arteritis when they died 8-17 days later. The lesions affected principally the elastic arteries, the commonest sites being the aorta, sinuses of Valsalva, coronary arteries, pulmonary arteries and the endocardium of the left auricle. These lesions are uniformly absent from the parenchyma of organs other than the heart and lungs.

All of the dogs which developed lesions were maintained on a standard low protein diet. Four dogs maintained on the regular kennel diet of meat scrap and subjected to the same experimental procedure failed to develop lesions. Likewise 5 dogs maintained on the standard low protein diet and receiving the same dose of uranium nitrate (5.0 mg. per kilo) but not subjected to plasmapheresis or plasma injection failed to show any arterial lesions.

Thus 3 factors seem to be essential for the production of these arterial lesions: 1. standard low protein diet, 2. plasma alteration, 3. heavy metal injury. (*See pp. 359-375 of this issue.*)

STUDIES OF THE POTENTIALITIES OF NORMAL AND PATHOLOGICAL HUMAN MONOCYTES *in Vitro*. By Ben C. Houghton. *From the Department of Pathology, Ohio State University College of Medicine, Columbus.*

Under cultural conditions the normal monocyte of the peripheral blood exhibits a wide variety of morphological patterns. In surface films during the first three days monocytes become highly phagocytic, ingesting many granulocytes and red cells. These forms become less motile, and during subsequent days increase in individual size. On the tenth day, the cytoplasm has lost its ingested material and the nucleus has become more vesicular. The stellate and fusiform character suggests a reticulum cell. At the same time many non-lymphocytic, non-phagocytic cells of the monocytic type lose the cytoplasmic and characteristic motile properties shown in the first forty-eight hours. These cells become spherical and acquire vesicular nuclei with large nucleoli. The cytoplasm develops a deeply basophilic staining property. By the tenth day, many mitotic figures may be seen among these cells and about the *Hof* of the nucleus, granulations appear. In fourteen days, cells indistinguishable from granular myelocytes, by supravital, Wright's-Giemsa or oxydase staining, are abundant. Mature granulocytes showing typical amoeboid motility reappear within three weeks. Patterns of variation and time intervals may be influenced to some degree by modification of media.

QUANTITATIVE ASPECTS OF THE PHAGE-ANTIPHAGE REACTION. By A. D. Hershey and J. Bronfenbrenner. *From the Department of Bacteriology and Immunology, Washington University School of Medicine, Saint Louis.*

When coliphage and homologous rabbit antiphage of suitable concentrations are mixed in various proportions, and the mixtures are allowed to stand two or three days at low temperatures, a stable condition is reached both with respect to amount of phage neutralized and the amount of neutralizing antibody adsorbed. As the initial proportions vary from about two to 50 antibody molecules per active phage unit, the corresponding degree of neutralization, as measured by plaque counts, varies from about 10 to 95 per cent of the original phage. Somewhat larger ratios lead to complete neutralization.

The reaction is accompanied by visible aggregation and, in incompletely neutralized mixtures, the residual phage activity is found in the sediments. It appears, therefore, that the reduction of plaque counts effected by the smaller amounts of antibody may be attributed, at least in part, to aggregation of active phage. This consideration indicates that the adsorption of about 50 antibody molecules results in the neutralization of a single phage, independently of aggregation. This figure is subject to two possible sources of error, however. It is based on an antibody determination by the method of Heidelberger and Kabat, using phage-coated bacteria as antigen, which we have not adequately controlled. It also assumed that the phage preparations contained no substances other than active phage which adsorb neutralizing antibodies.

It should be noted that the result obtained here neither contradicts nor confirms an earlier finding based on statistical analysis of the neutralization curve (*J. Bact.*, 1941, 41, 60). The latter is concerned only with those among the adsorbed molecules of antibody directly effective in neutralization.

By titration of neutral mixtures for residual neutralizing antibody, it is found that a single phage unit may adsorb up to 3,000 antibody molecules, and that amounts in excess of this remain free in the mixture. This figure does not vary appreciably with the final concentration of free antibody. It is, however, subject to the errors already mentioned.

These quantitative relations are not appreciably affected by 4-fold variations in the volume of solvent. Other attempts to demonstrate an equilibrium condition have so far led to indecisive results.

The ratio of the amount of antibody required to neutralize to the amount with which the phage can combine (1:60) is probably independent of the sources of error noted. It seems likely that the small value of this ratio may explain the superficial differences between the neutralization of phage and that of certain other viruses.

THE SIGNIFICANCE OF GLUCOSE AND NON-GLUCOSE-REDUCING SUBSTANCES IN POSTMORTEM BLOOD. By Edwin V. Hill. *From the Department of Legal Medicine, Harvard Medical School, Boston.*

The various factors which influence the rate and amount of change in the glucose and the non-glucose-reducing substances of blood after death were studied in experimental animals and in human subjects. It was found that

EXPERIMENTAL POLIOMYELITIS IN GUINEA PIGS. By Claus W. Jungeblut and Murray Sanders. *From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*

In previous experiments the mouse strain of SK poliomyelitis virus possessed no demonstrable pathogenicity for guinea pigs. Continued mouse transfers resulted in an increase of virulence from 1:1 million to 1:1 billion dilution activity. When examined again at the 70th mouse passage, the murine virus proved capable of paralyzing guinea pigs.

Approximately 90 per cent of the infected guinea pigs develop flaccid paralysis of the extremities following intracerebral injection with murine virus; the disease is also transmissible by feeding. The virus passes through all grades of Berkefeld filters. It may be recovered from brain or cord of paralyzed guinea pigs but not from blood; animals fed the virus discharge it in their feces.

Paralyzed guinea pigs present a typical poliomyelitic process in the spinal cord with perivascular infiltration, ganglion cell necrosis, and marked neuronophagia. Lesions in the white matter are rare; the meninges are normal.

Guinea pig passage virus is completely inactivated by guinea pig convalescent serum, antimurine rabbit immune serum and SK monkey convalescent serum; conversely, guinea pig convalescent serum neutralizes SK monkey virus. The serological evidence therefore suggests the identity of the virus in its three hosts, *i.e.*, monkey, mouse and guinea pig.

In the first guinea pig passages the incubation period was short (2 to 4 days); a gradual lengthening (7 to 21 days) occurred with subsequent transfers. Two attempts to maintain continuous serial passage in guinea pigs came to an abrupt end with the 5th and 6th passages, respectively. While active in guinea pigs, the infectious agent could easily be returned to mice and cotton rats but not to rhesus monkeys. When its virulence diminished for guinea pigs and mice, injection of a rhesus monkey with the cord of a paralyzed guinea pig produced classical poliomyelitis. It appears that a complete cycle of pathogenicity has been established for the SK strain from the monkey to cotton rats, white mice, guinea pigs and back to the monkey.

EVIDENCE OF SEROLOGICAL HETEROGENEITY OF POLYVALENT "PURE LINE" BACTERIOPHAGE. By G. M. Kalmanson and J. Bronfenbrenner. *From the Department of Bacteriology and Immunology, Washington University School of Medicine, Saint Louis.*

It is well known that a "pure line" phage may possess true polyvalence, that is, it may act equally well on several more or less related organisms, regardless of which organism was used for its propagation. It has been found earlier that such a phage (PC), acting on *B. coli*, *B. shiga* and *B. flexner*, is heterogeneous in the sense that by means of heat inactivation it was possible to destroy the valence for *Shiga* and *Flexner* and secure a weakly active "monovalent" phage for *B. coli*. In the present investigation we were interested in ascertaining whether the neutralizing antiserum prepared against a highly purified preparation of this polyvalent phage, and devoid of all demonstrable antibacterial agglutinins, would neutralize all three valences and, if it did so, whether it would be possible to detect by this procedure any heterogeneity corresponding to that previously demonstrated by heat inactivation.

Monocytes in relatively pure cultures have been obtained for similar study from patients with chronic monocytic leukemia. These cells produce a uniform growth of "fibroblast-like" character which may be carried through numerous passages, a phenomenon which is in sharp contrast to leukemic myeloid or lymphatic cell cultivations.

The monocyte of acute monocytic leukemia, on the other hand, shows little tendency to follow the usual pattern of cyclic variations, or to change from its undifferentiated state.

MACROMOLECULAR SUBSTANCES AS DISEASE-PRODUCING AGENTS. By W. C. Hueper. *From the Warner Institute for Therapeutic Research, New York City.*

The intravenous introduction of several chemically inert, non-toxic, osmotically active, macromolecular carbohydrates, suitable as non-hematogenous blood substitutes, gives rise, when injected in excessive concentrations and amounts, to disturbances of the colloidal, macromolecular equilibrium of the plasma leading to the development of a macromolecular hematological syndrome and of an intracellular and extracellular storage of the injected material in various organs. The hematological symptom complex consists of a reduction in the number of erythrocytes and amount of hemoglobin; initial, transitory leukopenia; persistent leukocytosis; increased conglutination and sedimentation speed of erythrocytes; thrombocytopenia or thrombocytosis; impaired coagulability; normal fragility of erythrocytes; normal or moderately lengthened prothrombin time; lengthened bleeding time; hemorrhagic diathesis; decrease in fibrinogen and total plasma protein. The macromolecular material is taken up and immobilized by reticuloendothelial and endothelial cells of small and large vessels, by mobile and fixed phagocytes as well as cells of parenchymatous organs (kidney, liver, brain, suprarenal, etc.) transforming them into foam cells causing functional and anatomical disturbances. The hematological reactions and organic changes of these experimental thesauroses resemble those seen in glycogenosis, amyloidosis, lipoidoses and immunity responses.

PRODUCTION OF ELONGATED (PENCIL) ERYTHROCYTES IN CHRONIC HEMORRHAGE. By Raphael Isaacs and Leonard D. Rosenman. *From the Department of Hematology, Michael Reese Hospital, Chicago.*

Elongated (pencil) erythrocytes are present in from 0.5 to 2.0 per cent of the cells in the peripheral blood of patients who lose small quantities of blood over a long period of time. The cells have an average length of 10.72 microns (9 to 12) and a width of 3.0 microns. The sides are parallel and the ends rounded. They appeared in the peripheral blood of dogs, rats and mice subjected to repeated bleeding. Their appearance varied in different animals from 18 days to 4 weeks, and, as in man, there appeared to be no correlation between the level of the red blood cell count and the appearance of the cells. No bone marrow changes were noted which could account for the peculiar shape of the cells. No changes were noted *in vitro*. The cells are diagnostic of chronic hemorrhage.

tinctive substance in the Brown-Pearce tumor (*J. Exp. Med.*, 1940, 71, 335), an antibody wholly distinct from the antiviral antibody just described has been encountered in the blood of rabbits carrying the V2 carcinoma. Its properties and affinities will be reported.

STUDIES ON THE INTRAVENOUS ADMINISTRATION OF BOVINE PLASMA TO MAN.

By A. J. Kremen, H. Hall, H. Koschnitzke, B. Stevens and O. H. Wangenstein. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The possibility of administering bovine plasma intravenously to man was described previously by Wangenstein, Hall, Kremen, and Stevens. It is proposed to report here on extended observations made since that time with reference to (1) the efficacy of the method, and (2) nitrogen balance studies and (3) effect of agglutinin adsorption with human red blood cells on the incidence of reactions.

GASTRIC ACIDITY AFTER VARIOUS TYPES OF OPERATION FOR ULCER IN MAN.

By Bernard Lannin and Owen H. Wangenstein. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Achlorhydria to histamine stimulation has been set up as a desirable eventuality after operation for ulcer in man. In order to evaluate the effect of operative procedure on gastric acidity, seven types of operations have been done: (1) Anastomotic procedures, (2) Antral resection with termino-lateral gastro-jejunostomy, (3) Extensive gastric resection including excision of antrum and pylorus with termino-lateral gastro-jejunostomy, (4) Finsterer exclusion operation (same as 3, but leaving pylorus and antrum intact), (5) Small gastric resection with complete intragastric regurgitation, (6) Extensive excision of acid secreting area, and (7) Same as 6 with addition of gastro-jejunostomy.

The effect of these various operations upon gastric acidity was discussed.

THE PATHOGENESIS AND PATHOLOGY OF EXPERIMENTAL TYPE I PNEUMOCOCCUS PNEUMONIA IN THE MONKEY. By Clayton G. Loosli. *From the Department of Medicine, University of Chicago.*

Fourteen healthy monkeys were infected intrabronchially with Type I pneumococci suspended in starch and killed at increasing intervals of time within 7 days after inoculation—six were killed within the first 24 hours, seven within the next 72 hours, and one at 7 days. Lobar consolidation began at the site of inoculation and progressed in a contiguous manner until complete at 24 hours. During this time the infection was confined principally to the alveolar and bronchial air spaces. The pneumococci spread through the lobe from the site of inoculation by extension of the infected edema fluid directly from alveolus to alveolus, through the pores of Kohn, and from bronchiole to bronchiole, by repeated aspiration as the result of breathing and coughing. The cellular exudate enters the alveoli through the capillaries in the alveolar walls, and changes during the first 72 hours from one of polymorphonuclear leukocytes to one of mononuclear macrophages, in spite of early persistent bacteremias. The mononuclear macrophages arise principally from the hypertrophy of hematogenous lymphocytes and monocytes, which migrate early into the exudate, and to a lesser extent, from the

The results of these experiments indicated that *Shiga* and *Flexner* valences may be completely neutralized by this serum before that for *coli*. When the resulting "monovalent" phage is propagated on *B. coli*, however, it immediately regains its polyvalence (*Shiga* and *Flexner*), as was found to be the case also with the "monovalent" phage secured by heat inactivation.

Statistical analysis of experimental data indicated that the observed difference in the rates of neutralization is not due to a difference in the number of antibody molecules required to neutralize one lytic unit of each valence. Therefore, the difference in the observed rates of neutralization seems to be qualitative rather than quantitative in nature.

THE RATE OF DISAPPEARANCE OF THE EASTERN EQUINE ENCEPHALOMYELITIS VIRUS FROM THE BLOOD OF RABBITS FOLLOWING THE INJECTION OF INDIA INK. By J. E. Kempf, M. E. Pierce and M. H. Soule. *From the Department of Bacteriology, University of Michigan, Ann Arbor.*

In series of 5 experiments, 19 rabbits were injected daily by the intraperitoneal and intravenous routes with a suspension of 7 per cent India ink (Higgins) in 0.85 per cent saline solution in an attempt to block the reticuloendothelial system. The efficiency of the procedure was determined on the third day by estimating colorimetrically the amount of Congo red in the serum one hour after injection. The removal of the virus of EEE from the blood stream following its intravenous injection into these animals and 21 controls was observed as follows: Blood was withdrawn from the ear vein at definite intervals and the presence or absence of virus was detected by mouse injection.

It was found that the rate of disappearance of virus from the blood was the same in the partially "blocked" animals as in the controls. However, the mortality rate from encephalomyelitis in the "blocked" rabbit was 94.7 per cent, while in the controls it was 33.3 per cent.

DISTINCT TYPES OF ANTIBODY IN THE BLOOD OF RABBITS CARRYING THE TRANSPLANTED V2 CARCINOMA. By John G. Kidd and William F. Friedewald. *From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*

Experiments already reported have shown that rabbits carrying the transplanted V2 carcinoma—a squamous cell carcinoma derived originally from a virus-induced papilloma—regularly develop in their blood an antibody capable of neutralizing *in vitro* the papilloma virus with which the growth was initiated, and capable also of fixing complement in mixture with it (*J. Exp. Med.*, 1940, 71, 813). Further studies have now shown that virus-neutralizing and complement-fixing titers of sera procured from rabbits carrying the V2 carcinoma invariably parallel one another, and that the antibody responsible for the two reactions can be readily absorbed from the sera upon admixture with the papilloma virus. From the findings it appears certain that the antibody is directed against the papilloma virus *per se* and that it is identical with the antiviral antibody present in the blood of rabbits carrying benign virus-induced papillomas and with that present in the blood of rabbits injected with purified papilloma virus (*J. Exp. Med.*, 1940, 72, 531).

In further serum tests carried out by the methods that disclosed a dis-

obstruction of the duodenum. The paucity of significant peritoneal pathological findings at autopsy has caused such deaths to be attributed usually to atelectasis or pneumonia. Mindful of the suggestion of C. A. Dragstedt and his associates that relatively low sustained intraluminal pressures in the duodenum may compromise the viability of the bowel, a comparable situation was established in the dog. Hyperthermia was a frequent complication. A satisfactory method of preventing the occurrence of this complication in man after gastric resection was described.

HYPOPROTEINEMIA TREATED BY PROTEIN DIGESTS BY VEIN. By Sidney C. Madden. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

Hypoproteinemia is produced in dogs by plasmapheresis. A casein digest given by vein will maintain such a hypoproteinemic dog in nitrogen balance and weight equilibrium for many weeks provided that adequate non-protein requirements are given by mouth or otherwise. Such a dog getting intravenous digest regains his normal plasma protein concentration when plasmapheresis is discontinued.

THE EFFECT OF HEPATECTOMY, AND ABDOMINAL EVISCERATION WITH AND WITHOUT HEPATECTOMY ON THE SERUM PHOSPHATASE OF THE DOG. By Stephen Maddock, Harry C. Trimble, Dorothy Jensen and William Appleby. *From the Boston City Hospital.*

In a series of dogs it has been found that total hepatectomy results in a rise of serum phosphatase to the neighborhood of 50 Bodansky units during the course of the experiment. The increase in serum phosphatase is not due to anesthesia, nor can it be accounted for on the basis of injected glucose, since glucose given hourly in amounts comparable to those needed to prevent hypoglycemia in the liverless dog causes no rise in phosphatase.

When all the abdominal viscera including the liver and kidneys are removed there is no increase in serum phosphatase in the early postoperative hours and only a slight terminal rise to the vicinity of 10 Bodansky units.

The removal of the entire gastro-intestinal tract, spleen and pancreas, leaving the kidneys and liver intact, causes no rise in serum phosphatase.

These results are interpreted as meaning that phosphatase coming from the intestines and possibly the kidneys and bone also, is utilized by the liver for the dephosphorylation of hexose-1-monophosphate (Cori ester). In the absence of the liver the enzyme accumulates in the blood.

The rise in phosphatase following hepatectomy is much more rapid than that found after common bile duct ligation since it ordinarily requires 5 to 7 days for the serum of these animals to reach a value of 50 Bodansky units. This gives some conception of the magnitude of the rôle of the liver in the utilization of phosphatase. These experiments suggest a method for the future study of the origin of phosphatase in the animal body, and a means of determining whether more than one type of alkaline phosphatase exists.

SEX HORMONES AND LYMPHOMATOSIS OF FOWLS. By David Marine and Samuel H. Rosen. *From the Laboratory Division, Montefiore Hospital, New York City.*

We have previously reported upon the occurrence of lymphomatosis in 24 of 56 White Leghorn capons and its absence in 61 slips of the same group—

septal cells in the alveolar walls. Interstitial involvement becomes pronounced only after consolidation is complete and is associated with complications such as septicemia, and not with the mechanism which brings about consolidation.

EXPERIMENTAL SQUAMOUS CELL CARCINOMA OF THE FORESTOMACH IN MICE AND THE METHOD OF INDUCTION BY ORAL ADMINISTRATION OF CARCINOGEN. By Egon Lorenz and Harold L. Stewart. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

When an olive oil emulsion containing either 20-methylcholanthrene or 1, 2, 5, 6 dibenzanthracene is administered to different strains of mice instead of drinking water, the lesion regularly observed in the gastrointestinal tract is adenocarcinoma of the small intestine; only in rare cases squamous cell carcinoma of the forestomach was induced. Squamous cell carcinomas of the forestomach can be induced in a high percentage of animals by stabilizing the olive oil emulsion with the dioctylester of sodium sulfosuccinate and in still higher percentage by substituting mineral oil emulsion for the olive oil emulsion. Data was given as to the composition of these emulsions, the amount of emulsion consumed by the animals, and the amounts necessary for the induction of the gastric tumors. The mechanism of the induction of these tumors was discussed.

EPITHELIOMA OF LIP IN CATFISH. I. PATHOLOGY OF SPONTANEOUS TUMORS. II. GROWTH OF INTRACORNEAL TRANSPLANTS. By Balduin Lucké and H. Schlumberger. *From the Laboratory of Pathology, School of Medicine, University of Pennsylvania, Philadelphia.*

Catfish from streams near Philadelphia have been found to be commonly afflicted with epithelioma. The tumors usually occur as large, red, fleshy masses on the lips or dental plates; sometimes they become so massive as to prevent closure of the mouth. In approximately one-third of the cases, secondary tumors develop on that part of the opposite lip in direct contact with the primary growth. Histologically, the neoplasms consist of epithelial cells, often in papillary arrangement; the larger tumors commonly extend downward, invade adjacent tissues, and push into vessels in which tumor emboli are frequently found. Experimentally, we have transmitted the tumor to fish of the same species by implanting it between the layers of the cornea (which in catfish are readily separable). Here the growth of the transplants has been followed by periodic examination of the living tumors with the slit lamp microscope. It was thus learned that this cancer develops according to a definite structural pattern, namely, in the form of undifferentiated membranes which gradually thicken and become compact. In this manner growth continues until the tumor fills the cornea.

HYPERTHERMIA AND DEATH CAUSED BY A CLOSED DUODENO-JEJUNAL LOOP AFTER GASTRIC RESECTION; PREVENTION OF THE COMPLICATION; EXPERIMENTAL REPRODUCTIONS OF THE SYNDROME IN THE DOG. By David Lynn, Lyle J. Hay and Owen H. Wangenstein. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Hyperthermia and death have been observed in man, as complications of gastric resection, as well as of gastro-jejunostomy performed for high-grade

PULMONARY ARTERY TO LEFT AURICLE ANASTOMOSIS WITH HYPERTROPHIC OSTEOARTHROPATHY. By M. Mendlowitz and A. Leslie. *From the Laboratories of the Mount Sinai Hospital, New York City.*

By a special technique the pulmonary artery was successfully anastomosed to the left auricle in four dogs. The cardiac output (Fick method), blood volume, arterial and venous blood pressures, ether and cyanide circulation times were measured before and one or more times after operation in three of these dogs. The per cent shunt was also calculated. Two dogs died three months and three weeks, respectively, after operation of intercurrent infection (pneumonia). The first of these dogs had a 46 per cent shunt and developed a moderate secondary polycythemia and a systemic cardiac output in excess of the preoperative. The blood flow through the lungs was normal. The cyanide circulation time and the arterial blood pressure were decreased but the oxygen consumption and all the other determinations remained unchanged. The other dog had an obviously large shunt (cyanosis) but died before postoperative studies could be carried out. The remaining two dogs are still alive 9 and 7 months, respectively, after operation and are in good condition. In the former there is a 13 per cent shunt, a somewhat decreased cyanide circulation time, an excessive systemic cardiac output but no other changes. In the latter there is a 17 per cent shunt, a short cyanide circulation time, and excessive systemic cardiac output and bone changes indistinguishable by X-ray from hypertrophic osteoarthropathy. The only consistent circulatory change and probable cause for this bone condition is a systemic cardiac output in excess of tissue needs.

ON THE MECHANISM OF ENHANCED DIABETES WITH INFLAMMATION. By Valy Menkin. *From the Department of Pathology, Harvard Medical School, Boston.*

A study has been undertaken in an effort to determine the factors concerned in influencing the state of diabetes with inflammation while at the same time the inflammatory reaction appears concomitantly to be intensified. In brief, it has been found that in the depancreatized dog with inflammation there is an enhancement in the degree of diabetes as indicated by the marked rise in blood sugar. There is likewise a concomitant increased proteolysis at the site of inflammation. This is indicated by the rise in urea, non-protein nitrogen, and amino acid nitrogen of exudates. The rise in the concentration of sugar and lactic acid of exudates seem to be referable, at least in part, to the increased proteolysis favoring thus, by deamination, the conversion of part of the protein molecule to glucose. The conversion of protein or amino acids to glucose and lactic acid is known to occur in the diabetic animal as described in the earlier work of Lusk and of others. Tissue injury is essentially the result of proteolytic processes. An inflamed area is a focus of proteolysis. The hypothesis advanced by the writer is that such foci manifest an intensified degree of proteolysis in the diabetic organism. Thus the enhanced local proteolysis in a diabetic animal, either incapable of burning its own glucose or else producing an over-abundance of this substance, would both intensify tissue damage by protein degradation and at the same time favor local gluconeogenesis. Furthermore, studies also indicate that insulin administration inhibits not only the more severe manifestation of diabetes, but also the enhanced proteolysis at the site of inflammation.

19 of which were older than the youngest bird that developed the disease. This observation suggested that the presence of a functionally active fragment of testis may have inhibited the development of the disease—possibly because of the production of sex hormones.

The present experiment includes 41 White Leghorn male castrates, divided into 3 groups as follows: 1) 12 birds, each receiving 1 mg. of estradiol dipropionate, weekly; 2) 12, each receiving testosterone dipropionate in variable amounts twice weekly; and 3) 17 controls. Injections of these hormones were started on June 18, 1940, and they are still being given to the survivors.

To date (January 15), 4 or 33⅓ per cent of the androgen group, 7 or 58 per cent of the estrogen group and 4 or 23.5 per cent of the control group have died of lymphomatosis. The youngest (receiving estradiol) was 201 days old and the oldest to date, 319 days. Routine biopsies have shown that the liver may be transformed from a normal appearing organ to a huge reddish gray organ in 15 days.

In only one of the 15 cases was a fragment of testis found at autopsy—confirming our observation in the first series.

Anatomically the cases may be grouped as 1) diffuse hepatorenal (big liver disease) type—8 cases; 2) lymphosarcoma—4 cases; 3) lymphoma of the bursa of Fabricius—2 cases; and 4) neurolymphomatosis—1 case. The incidence of lymphomatosis in the androgen group may not be significant since the dose of androgen during the first 4 months of the experiment varied from excessive to a submaintenance level. The occurrence of lymphomatosis in 58 per cent of the estrogen group as opposed to 23.5 per cent of the control group suggests that the induced sex hormone imbalance may have been a factor in stimulating or activating the agent responsible for overgrowth of lymphoblastic tissue.

PERIPHERAL VASCULAR REACTIONS IN ANAPHYLACTIC SHOCK OF THE MOUSE.

By Philip D. McMaster. *From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*

The vascular reactions in the ear of the mouse have been studied under high power during anaphylactic shock produced by a highly colored antigen, the proteins of horse serum coupled to an azo dye, T-1824, by diazotization.

After the antigen has been injected into the blood, local or general constriction of veins often occurs either before or after the arterial spasm, and sometimes together with it. If venous spasm precedes arterial spasm, the capillary bed becomes choked with cells; if it occurs later, the tissues become bloodless. No constriction of capillaries has yet been observed; the capillary behavior seemed to follow passively the changes in the large vessels.

Peripheral vascular spasm occurs while the carotid blood pressure is high and none of the colored antigen can be perceived in the vessel endothelium. Following the vascular reaction the blood pressure falls. The lymphatics undergo no change nor is there any in the interstitial pressure.

If the circulation of one ear is obstructed during anaphylactic shock, no vascular spasm occurs in it. Release of the obstruction, during the animal's recovery, results in belated constriction of the blood vessels, although now the vessels in the other ear are dilated and the general blood pressure is low.

The vascular reactions in the ears are not the consequence of nervous stimuli but are local in origin, and are uninfluenced by the blood pressure.

litters of susceptibles contained 95 rats, and of these 94 showed one or more progressively growing takes (99 per cent). Each rat received four implants except 10 which received only two. Of the 360 implants made, 343 grew (95 per cent). The seventeen resistant litters contained 110 rats, and of these 40 showed one or more progressively growing takes (36 per cent). Each rat received four implants except 19 which received only two. Of the 402 implants made, 82 grew (20 per cent). The tumor had been stabilized previously by passing it through 15 transplant generations. Although the experiment is not completed, the selection seems to have produced two hereditary lines which exhibited marked difference in susceptibility to tumor implantation. A recent complication has arisen in that most of the resistant litters have failed to reproduce.

EFFECTS OF DIETARY YEAST ON DEVELOPING AND HEALING CARBON TETRACHLORIDE CIRRHOSIS IN THE RAT. By Joseph Post, David P. Earle, Jr. and Joseph Victor. *From the Research Service, First Division, Welfare Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.*

The influence of feeding various amounts of yeast on CCl_4 cirrhosis was studied. When the food intake was restricted to the same level in all animals, there was no difference in the development of cirrhosis between rats fed large amounts or minimal amounts of yeast needed for normal growth. Likewise the healing of cirrhosis was the same in both groups. However, when the food intake was uncontrolled, cirrhosis was more severe in rats fed yeast in amounts below that necessary for normal growth.

CCl_4 injections markedly inhibited the growth of rats regardless of the yeast intake. The poor growth of rats developing or recovering from CCl_4 cirrhosis of the liver was improved by the feeding of yeast in excess of the normal rat requirements. A similar growth acceleration was not observed by feeding comparable amounts of yeast to uninjected controls. High yeast feeding exerted a limited lipotropic effect on the fatty infiltration of the liver resulting from CCl_4 administration.

THE DEVELOPMENT OF THE AGENT OF LYMPHOGRANULOMA VENEREUM IN THE YOLK SAC OF THE CHICKEN EMBRYO. By Geoffrey Rake, Helen Jones and Morris F. Shaffer. *From the Squibb Institute for Medical Research, New Brunswick, N.J.*

The agent of lymphogranuloma venereum, when introduced into the yolk sac of the developing chicken embryo, produces an infection fatal to the embryo in from 3 days to 2 weeks. Studies on the distribution of the virus by transfer to new series of embryos by the yolk sac route show that the virus is present in large quantities in the wall of the yolk sac and in the yolk itself. The development of the agent in the wall of the yolk sac has been investigated by means of histological sections and alcohol-fixed impression smears. The pathological picture consists essentially of the development, within the yolk cells, of small foci of dark-staining bodies which increase rapidly in number and, concurrently, decrease somewhat in size until the cytoplasm of the distended yolk cell is filled with small particles. These

The available evidences, therefore, support the view that the mechanism of enhanced diabetes with concomitant inflammation might well be referable primarily to an increased local proteolysis in the inflamed area, favoring a combined picture of increased tissue damage with a corresponding elevation in glucose formation; the glucose, in turn, gradually diffuses into the systemic circulation.

LESION PRODUCED IN THE BONE MARROW BY INJECTION OF SPECIFIC ANTIBODIES. By Anderson Nettleship. *From the Department of Pathology, Union University, Albany Medical College.*

Antibodies capable of producing widespread changes in the bone marrow were obtained by injecting extracts of bone marrow or leukocytes into an heterologous species. These extracts were prepared by grinding rabbit bone marrow or leukocytes, and following a preliminary fat extraction the protein was precipitated with abundant cold acetone. The water soluble precipitate was injected twice weekly into guinea pigs for a period of eight weeks. Ten days after the last injection the guinea pigs were bled out and their serum pooled. Five to ten milliliters of this serum was injected intravenously into rabbits. In acute experiments the peripheral blood and bone marrow were studied prior to and following a single injection. In chronic experiments the same technique was used, following multiple injections, over a period of six weeks. The results showed a dramatic drop in circulating polymorphonuclear leukocytes within ten minutes following the injection. This persisted for as long as twenty-four hours. Sections of bone marrow taken within seventy-two hours following the injection showed fresh necrosis and hemorrhage in small irregular shaped areas throughout the bone marrow cavity. The chronic preparations showed not only areas of necrosis but also areas of marked hyperplasia. There were also many cysts and areas of heavy fibroblastic proliferation. These latter bear a striking resemblance to osteitis fibrosa as seen in humans.

INFLUENCE OF BREEDING ON SUSCEPTIBILITY OF WHITE RATS TO TRANSPLANTATION OF A METHYLCHOLANTHRENE SARCOMA. By J. Lowell Orbison, H. A. Davenport and Frank B. Queen. *From the Department of Anatomy and the Patterson Laboratory for Cancer Research, Northwestern University Medical School, Chicago.*

A spindle cell rat sarcoma (our No. R 86), which was induced by methylcholanthrene, failed to grow in some litters, while nearly all implants grew in other litters. Assuming this to be a manifestation of heredity, breeding experiments were begun to attempt a purification of the factors responsible. The procedure used to separate the resistant and susceptible lines was a selection of pairs which had been tested by transplanting when they were about two months old. As the two lines were developed, the value of a particular mating was determined by transplanting the entire litter of offspring, and using only those individuals for further breeding which produced or were part of completely resistant or susceptible litters. Susceptible pairs were saved for breeding after total extirpation of the growing tumors. Between September 1, 1940 and December 20, 1940, seventeen litters of each line have been transplanted with the tumor to test their responses. The seventeen

fasting ascorbic acid concentrations of 0.1 to 0.2 mg. per cent represent distinctly less marked depletion, evidence of the type set forth below, indicates that they are probably clinically significant.

The absolute diagnosis of subclinical scurvy should be based upon some objective or clearly defined subjective change resulting from administration of vitamin C in cases suspected of deficiency. Two useful objective tests are determination of the capillary resistance and study of erythropoietic responses. Using these criteria it is shown that plasma ascorbic acid levels below 0.2 mg. per cent are usually indicative of clinically significant deficiency. Values of 0.3 to 0.5 mg. per cent probably do not indicate significant deficiency even though the saturation levels are considerably higher, *ie.*, 1.0 to 1.4 mg. per cent.

COMPARISON OF HEMOGLOBIN PRODUCTION IN EXPERIMENTAL ANEMIA OF SEVERE AND MODERATE DEGREE. By F. S. Robscheit-Robbins. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

These studies concern an experimental hemorrhage-anemia of varying degrees of severity in dogs. The effect of a variety of diet factors on hemoglobin production is determined in an anemia of severe type as represented by hemoglobin levels of approximately 6 gm. and in anemia of more moderate degree with hemoglobin levels approximating 10 gm. The response to diet factors as concerns hemoglobin production decreases as the anemia becomes less severe. A comparison of results obtained was made.

STUDIES ON THE RELATION OF "NEUROTROPIC" STREPTOCOCCI TO POLIOMYELITIS AND ITS VIRUS. By Edward C. Rosenow. *From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.*

The studies here reported represent attempts to find the probable nature of the relation of the more or less characteristic cultivatable bacteria which are associated commonly with virus diseases. The streptococci were isolated from poliomyelitic and other material by the use of mediums especially favorable for growth of streptococci. Pure cultures of streptococci, far removed from virus, were grown in autoclaved infantile tissue consisting of the contents and shells of nineteen-day hatching eggs passed through a meat chopper (one part) and distilled water (seven parts). After growth for a variable time in this medium, extremely small pleomorphic forms appeared and a virus phase of the streptococcus sometimes developed. Virus "takes" were obtained first in mice and then after successive passages, in monkeys. The symptoms and lesions at first are chiefly encephalitic but after successive passage through monkeys, they become typical of poliomyelitis. The experimentally produced virus strains are filtrable and transmissible in series, and induce characteristic disease in extremely high dilutions. Monkeys that have recovered from natural virus are resistant to the experimental virus. Neurotropic streptococci were isolated with the same difficulty as from "natural" virus. Only strains that have neurotropic cataphoretic velocity, virulence and serologic properties, regardless of original source, have yielded virus.

bodies are apparently identical with the granulo-corpuscles of Miyagawa and appear to be elementary bodies of the agent. Definite correlation has been found between the development of the virus as shown by pathological preparations, and titrations of the yolk sac for infectivity.

THE LIVER GLYCOGEN CONCENTRATION FOLLOWING INTRAVENOUS GLUCOSE ADMINISTRATION AND DIET ON THE DOG AND MAN IN THE PRESENCE OF LIVER INJURY. By I. S. Ravdin, Harry M. Vars, Elizabeth Thorgood and Julius Schultz. *From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia.*

It is generally believed that high concentrations of hepatic glycogen protect the liver from many hepatotoxic agents. Regardless of the validity of this impression, it is also widely believed that glucose administered intravenously is the best method of increasing hepatic glycogen. This is untenable except for brief periods of time, since it disregards the calorific requirements of man or animal. We have studied this in the dog in the presence of common duct obstruction.

During voluntary feeding animals lost hepatic glycogen and increased liver lipid concentration. When in addition glucose was administered intravenously, the glycogen concentration was maintained and the liver lipid concentration was decreased. Where appetite was stimulated and the dogs received glucose intravenously, the liver glycogen concentration increased by 84 per cent and the lipid concentration decreased by 25 per cent. Following forced feeding (88 calories per kilogram of body weight per day) the liver glycogen concentration increased by 236 per cent and the lipid concentration decreased by 73 per cent. With only a 5 per cent solution of glucose intravenously (50 cc. per kilogram of body weight per day) the animal lost 50 per cent of the original liver glycogen concentration.

In man similar data have been obtained. The data from man and the dog suggest that if large accumulations of glycogen are desirable these must be obtained by the oral administration of foodstuffs, for such stores cannot be maintained unless the energy requirements are being met.

THE DETECTION OF MILD OR SUBCLINICAL SCURVY. By James F. Rinehart. *From the Department of Pathology and Medicine, University of California Medical School, San Francisco.*

This study is directed at the detection of mild or subclinical scurvy. It is based upon the correlation of many observations made during the past 5 years.

Fasting blood ascorbic acid concentrations have been correlated with curves of plasma concentration and urinary excretion following the ingestion of a large dose of ascorbic acid (15 mg. per kilogram). These data indicate that the character of the curve and the degree of undersaturation can be reasonably accurately predicted from the fasting blood concentration. Plasma ascorbic acid values of 0 to 0.1 mg. per cent usually indicate marked tissue depletion. The curves are usually flat and there is no urinary excretion. In a number of such cases where it has been possible to determine accurately the ascorbic acid deficit it has been found to be in the range of 3 to 4 grams. This is the approximate deficit existing in clinical scurvy. While

primary size 79 per cent; total metastatic weight 65 per cent; average metastatic weight 60 per cent; per cent mortality from carcinomatosis 50 per cent; animals with metastatic involvements 80 per cent; degree of metastasis 50 per cent. The differences are significant statistically.

Two experiments using insulin and thyroxin together made use of 60 animals (35 control, 25 treated). Comparison of the two groups indicates: total weight 21 per cent; metastatic weight 11 per cent; total tumor per animal 27 per cent; number metastases per animal 16 per cent; total number of metastatic involvements 34 per cent; total number of involvements per animal 50 per cent; metastatic involvements alone per animal 45 per cent; total severity (average weight at a locus times average occurrence of that locus) 26 per cent; metastatic severity 7 per cent. The differences are highly significant statistically.

The sixth experiment involving 60 animals is still in progress.

SOLUBLE ANTIGEN IN LYMPHOGRANULOMA VENEREUM. By Morris F. Shaffer and Geoffrey Rake. *From the Squibb Institute for Medical Research, New Brunswick, N.J.*

Previous workers have concluded that the Frei test demonstrates an allergic reaction due to the presence of the elementary bodies of the virus in the material injected. Thus it has been found that (a) with certain Frei antigens high-speed centrifugation yields non-reactive supernatants and dermally active sediments; (b) the passage of such Frei antigens through filters such as Berkefeld and Seitz K greatly reduces or completely abolishes the dermal activity.

With the aid of rich suspensions of virus, propagated in the yolk-sac of the chicken embryo or in mouse-lung, we have reinvestigated the problem. We have found that centrifugation of such suspensions at 12,000 RPM results not only in the deposition of virus-containing sediment but also yields a supernate which, despite its depletion in active virus, after further passage through Seitz EK discs still retains considerable ability to elicit a positive Frei reaction and to fix complement specifically in the presence of human lymphogranulomatous serum. Attempts to filter rich yolk-sac virus suspensions through Seitz EK filters result in filtrates with an infectivity reduced usually to zero or at least by more than a millionfold but with eight to fiftyfold diminished ability to elicit Frei reactions and to fix complement specifically. For such filtrates, therefore, antigen is present not in the form of elementary bodies but in a less highly organized, presumably soluble, form.

THE L-S SOLUBLE ANTIGEN OF VACCINIA. By T. Shedlovsky and J. E. Smadel. *From the Department of Medicine, The Hospital of The Rockefeller Institute for Medical Research, New York City.*

The heat labile (L) and heat stable (S) soluble antigens of vaccinia generally occur as a complex substance (L-S), (Craigie and Wishart). Several experiments (Parker, Craigie and Wishart) suggest that occasionally the complex may be dissociated, leaving free L and free S antigens.

Appreciable amounts of pure L-S complex were obtained from dermal filtrate by physico-chemical methods. Studies on such preparations by electrophoresis and also in the ultracentrifuge revealed only one component. The solutions precipitated to equal titer with L and S antibodies.

STUDIES IN MELANURIA. By Stephen Rothman. *From the University of Chicago.*

In 1935 Linnel and Raper showed that the chromogen in the urine of patients with malignant melanotic tumors is a simple derivative of 5-6 dihydroxy-indole which is also formed in the tyrosine-tyrosinase reaction by intramolecular rearrangement of catechol derivatives.

In clinical medicine the only qualitative chemical reaction used for detection of chromogens in melanuria is the darkening of the urine by addition of ferric chloride. This reaction is neither very sensitive nor specific enough, and in the case of dark urines its result is often doubtful. The findings of Linnel and Raper have been utilized in a case of melanuria (Service of Dr. G. F. Dick) by applying a series of sensitive qualitative color reactions which are characteristic for the chromogens (Thormählen, Ehrlich, nitroso-indol reaction) and to differentiate them from other indole compounds occurring in the urine such as indole and indican. Simple fractioning procedures were described for accumulation of the chromogen in order to increase the sensitivity of its qualitative color reactions.

EVIDENCE FOR DIFFERENT MECHANISMS IN CARCINOGENESIS BY HYDROCARBONS AND ULTRAVIOLET IRRADIATION. By H. P. Rusch and B. E. Kline. *From the McArdle Memorial Laboratory, University of Wisconsin, Madison.*

An attempt was made to determine whether the mechanism of carcinogenesis induced by ultraviolet irradiation is similar to that caused by the hydrocarbons. Ultraviolet irradiation of albino mice for thirty minutes daily over a period of four to five months resulted in a high percentage of ear cancers. The concurrent application of various carcinogenic hydrocarbons to the ears did not accelerate tumor formation. In other experiments mice were irradiated for periods of two to three months and hydrocarbons were applied to the irradiated parts for two months either preceding or following the irradiation. Each of these treatments was by itself just sub-carcinogenic. If the two treatments had supplemented one another, as the hydrocarbons do, cancers should have developed. However, no synergistic effect was observed and hence it is unlikely that the exposure of living tissue to ultraviolet irradiation results in the formation of carcinogens similar to the known hydrocarbons. Benzene extracts of irradiated skin were non-carcinogenic.

THE EFFECT OF INSULIN AND INSULIN PLUS THYROXIN ON THE METASTASIS OF THE BROWN-PEARCE EPITHELIOMA. By R. Ryer and J. R. Murlin. *From the Department of Vital Economics, University of Rochester.*

Six experiments were conducted. Three are concerned with the administration of insulin alone, two with insulin and thyroxin administered together, and one with thyroxin alone.

Young inbred New Zealand white bucks were inoculated with a sterile saline, cell suspension of the tumor. Death time was equalized by paired killing. All tumor was removed, weighed and separated into primary and individual metastasis in the last four experiments.

In experiments with insulin alone 84 animals were used (46 control, 38 treated). In summary (expressing insulin-treated as per cent of control):

FATTY CHANGES IN THE GLOMERULI OF THE KIDNEYS. By James P. Simonds and Jack D. Lange. *From the Department of Pathology, Northwestern University Medical School, Chicago.*

The glomeruli were examined for fat in Sudan-stained sections of the kidneys of 37 control dogs, of 96 dogs that had been injected intravenously with minute doses of colloidal poisons (bacterial toxins) and crystalline poisons such as potassium dichromate, and of 76 humans with varying types of nephropathies. Fatty changes may occur in the glomeruli in acute infectious diseases, in acute and chronic glomerulonephritis and in nephrosclerosis. In acute infections and in glomerulonephritis the fat is most abundant in the immediate vicinity of the glomerular hilus and the afferent artery is free from fat. In nephrosclerosis the afferent arteries contain much fat which stops at the glomerular hilus. The fat in the glomeruli appears first in the periphery, the central part being involved only in those in which fat is very abundant. In the first group, it is believed that the fatty changes are due to direct injury to the cells by a toxin circulating in the blood and concentrated in the glomeruli by loss of water by filtration; in nephrosclerosis, they are believed to be the result of ischemia due to narrowing of the lumen of the afferent artery.

THE INFLUENCE OF THE CONCENTRATION OF VIRUS PARTICLES ON INFECTION WITH VACCINIA. By Douglas H. Sprunt. *From the Department of Pathology, Duke University School of Medicine, Durham, N.C.*

Experiments have been done which show that the ability of vaccinia to produce a lesion is dependent not only on the amount of virus injected but also on the volume of tissue the virus reaches shortly after injection. The larger the volume of tissue the virus comes in contact with, the greater the chance for infection. In other words, if 10 virus particles are distributed through a tissue volume of 4 cc. their chance of producing a lesion is better than if these 10 virus particles were distributed through only 2 cc.

These conclusions have been reached by the following experiments:-

1. Experiments in which the volume of inoculum is varied. For example, it is shown that 10 virus particles in 0.1 cc. are no more likely to cause an infection than 5 virus particles in 0.5 cc.

2. Experiments in which the inoculum is kept localized with the estrogenic hormone. For example, if 10 virus particles are kept localized within a volume of tissue of 2 cc. their chance of producing an infection is no greater than if 5 virus particles are spread through 4 cc.

3. Experiment in which the virus particles were greatly dispersed with a spreading factor. In this experiment it is shown that the increased dispersion greatly increases the likelihood of infection.

These experiments not only show the difficulty of obtaining precise information from virus titration in animals but also emphasize again the inherent parasitic nature of viruses.

THE INFLUENCE OF ACTIVE AND INACTIVE ANTI-ANEMIC PRINCIPLES UPON THE SIZE OF THE ERYTHROCYTES OF OPOSSUMS (*Didelphis virginiana*) IN THE MATERNAL POUCH. By Joseph Stasney and Edward L. Burns. *From the Department of Pathology and Bacteriology of the Louisiana State*

Solutions of pure L-S antigen after heating precipitate with S antibody but not with L antibody; combination with L antibody still occurs, however, as shown in inhibition tests. L-S antigen treated with heat and dilute alkali completely loses all serological activity with L antibody and no longer precipitates with S antibody, but does inhibit it. These observations on pure L-S antigen confirm earlier results from our laboratories obtained with crude dermal filtrate.

It appears that L-S antigen is a molecular structure with two antigenically distinct parts, L and S. An hypothesis based on degradation of the S portion without change in the L portion of the molecule could explain the very occasional observations, which other workers have interpreted to indicate dissociation of the complex, into free L and S antigens.

TESTICULAR TUMORS IN MICE INJECTED WITH STILBESTROL. By Michael B. Shimkin and Hugh G. Grady. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Male mice of strain C (Bagg albino), three months of age, received a single subcutaneous implantation of a 6 to 8 mgm. cholesterol pellet containing 25 to 50 per cent of diethyl stilbestrol. Enlargement of the testes, usually unilateral, was noted about six months later. Within eight months approximately one-third of the animals have developed primary testicular tumors. The growths were composed of broad sheets of polygonal cells and appeared locally invasive. Large quantities of yellow pigment and moderate quantities of stainable fat were present. Necrosis was a prominent feature.

PARASYMPATHETIC EFFECTS OF PHOSPHOLIPIDS AND ERYTHROCYTES. By Gregory Shwartzman, M. B. Bender and E. Wachtel. *From the Mount Sinai Hospital, New York City.*

Laked erythrocytes injected intravenously or erythrocytes hemolyzed in the blood stream produce parasympathetic effects in the cat. Preliminary studies suggest that the substance producing these effects is associated with the phospholipid of the red corpuscles. The lecithins and cephalin extracted from human red blood cells, several vegetable and animal lecithins and synthetic lecithins have the same pharmacologic properties as the corpuscles, *viz*: when injected intravenously in aqueous emulsion each constricts the completely denervated iris of the cat but not that of the dog, monkey or rabbit; each produces a drop in the blood pressure of the cat, dog, and rabbit, but very little in the monkey. The synthetic lecithin is the most potent of the phospholipids tested. The isolated guinea pig ileum contracts with small quantities of lecithin and cephalin. Egg lecithin, cholesterol and gum acacia have no miotic effects.

The parasympathetic effects of the erythrocytes, lecithins and cephalin are not due to choline because none of these substances is blocked by atropine nor is any of them potentiated by eserine. Furthermore cephalin does not contain a choline radical. Recently we tested a compound in which the choline was excluded from the synthetic lecithin variety (distesoglycerophosphoric acid). This compound is more potent than the synthetic lecithin. It is inferred that the parasympathetic effects obtained by erythrocytes are due to the phospholipid in the blood.

In addition a number of the animals with changes in the stomach had adenocarcinoma of the small intestine. The mucosa of the pyloric chamber of the stomach was not involved except in certain cases in which the neoplasm in the cardiac chamber overhung somewhat the limiting ridge separating the two gastric chambers. The carcinomas of the forestomach were all of the squamous cell type. They extended through all coats of the gastric wall, formed nodular masses on the peritoneal surface and showed infiltration of or metastases to the following: mesentery, pancreas, mesenteric lymph nodes, genital omentum, spleen, diaphragm, lung, chest wall and posterior abdominal wall. Lantern slides illustrating the gross and microscopic features of these lesions were demonstrated.

LYMPH PROTHROMBIN. By S. A. Walker and K. M. Brinkhous. *From the Department of Pathology, State University of Iowa College of Medicine, Iowa City.*

In an effort to obtain further knowledge concerning the distribution of prothrombin in the body, its concentration in lymph collected from various sites was studied. Twenty-one normal dogs were used. The prothrombin content of thoracic duct, femoral and hepatic lymph was determined by the method of Warner, Brinkhous and Smith. It was found that the prothrombin content of liver lymph was nearly equal to that of dog plasma, while the prothrombin content of femoral lymph was less than one-tenth that of plasma. The prothrombin values for thoracic duct lymph varied widely, averaging about 50 per cent of the dog's plasma prothrombin. Fibrinogen determinations done at the same time on thoracic duct lymph averaged 51 per cent of the plasma fibrinogen. These results as a whole correspond closely to those which have been obtained for total proteins in lymph.

THE BEHAVIOR OF CELLULAR PROTEINASES (CATHEPSINS) IN EXPERIMENTAL TUBERCULOSIS OF RABBITS. By Charles Weiss. *From the Research Laboratories of the Mount Zion Hospital, San Francisco.*

In recent years there has been an increasing tendency to study the mechanism of allergy and immunity in tuberculosis from the viewpoint of the phenomenon of inflammation. Because of the important rôle played by the leukocytic and tissue proteinases in this process, we have studied the behavior of cathepsins in experimental tuberculosis of rabbits. It was observed that there is a marked depression of splenic cathepsin in animals which had been rendered tuberculin positive by repeated injection with a strain of low virulence and then reinfected with a virulent bovine strain of *M. tuberculosis*. Similar depressions in cathepsin were noted in the livers of these animals, but not in the kidneys or lungs. Reinfection with a strain of low virulence gave negative results.

It is of interest to note that the spleens and livers which present decreases in cellular cathepsins are, in Lurie's experiments, the most efficient in destroying virulent tubercle bacilli.

In vitro experiments demonstrated an inhibitory action of the phosphatide fraction of tubercle bacilli on cathepsins. It is suggested, therefore, that the destruction of virulent tubercle bacilli *in vivo* liberates phosphatide which, in turn, inhibits the activity of tissue cathepsins.

The probable relationship of these phenomena to the processes of caseation and softening of tuberculous tissue was discussed.

University School of Medicine and the Department of Pathology of the Charity Hospital of Louisiana, New Orleans.

Because of the similarity of erythrocytes in the mammalian fetus and in patients with pernicious anemia, attempts were made to influence the rate of maturation of the fetal red blood cells by administering anti-anemic principles. The opossum (*Didelphis virginiana*) was found to be a satisfactory species for this purpose because intra-uterine life lasts only about ten days, after which the immature young live and are easily accessible in the maternal pouch for 90 days.

In 117 immature opossums in the maternal pouch the mean maximal diameter of the erythrocytes was greater and the mean corpuscular volume larger than these respective values in ten adult animals of the same species. The direct injection of either normal human gastric juice or of a concentrated solution of liver extract into 38 immature pouch opossums significantly reduced the mean maximal diameter and the mean corpuscular volume of the red blood cells as compared with 27 untreated litter mate controls. These changes were effected through a relative increase in the number of smaller erythrocytes. The direct injection of either inactivated normal human gastric juice or of an inactivated concentrated solution of liver extract into 26 immature pouch opossums did not significantly reduce the mean maximal diameter and the mean corpuscular volume of the red blood cells as compared with 18 untreated litter mate controls. Gastric juice obtained from a patient with Addisonian pernicious anemia also failed to produce a significant reduction in the mean maximal diameter and in the mean corpuscular volume of the red blood cells of five immature pouch opossums as compared with three untreated litter mate controls,

THE NON-SAPONIFIABLE LIPID FRACTION OF LIVER FROM CANCEROUS AND NON-CANCEROUS PERSONS. By Paul E. Steiner. *From the Department of Pathology, University of Chicago.*

The amount of total non-saponifiable lipids recovered from the livers of thirty-three persons having cancer, eleven with various non-neoplastic diseases, and eight with cirrhosis of the liver, show no significant differences. The individual difference is enormous, varying from 0.32 per cent to 2.78 per cent, and it is not correlated with the type of tumor, the location of the primary tumor, or the amount of fatty change (fatty degeneration and fat infiltration) visible on microscopical sections. The fraction is of interest because it has cancerogenic properties.

MORBID ANATOMY OF INDUCED TUMORS OF THE FORESTOMACH IN MICE FOLLOWING ORAL ADMINISTRATION OF CARCINOGEN. By Harold L. Stewart and Egon Lorenz. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Mice which have received 20-methylcholanthrene orally as described in another abstract (see Egon Lorenz and Harold L. Stewart, "Experimental squamous cell carcinoma of the forestomach in mice and the method of induction by oral administration of carcinogen") showed the following lesions of the forestomach, singly or in combination: hyperkeratosis and hyperplasia of the mucosa, single or multiple papillomas, and squamous cell carcinoma.

central nervous system the cell bodies may exhibit varying degrees of chromatolysis and eccentricity of the nuclei. With the exception of some cellular infiltration in the peripheral nerves and sensory ganglia, no inflammatory reaction has been observed in the nervous system. The etiology of this form of polyneuritis is unknown, but, despite the fact that an early report² of transmission to monkeys has not been confirmed, the statement is often made that the disease is caused by an unknown filtrable virus.

The present study was begun with the intention of obtaining some orienting data concerning the etiology of the disease. All the previous fruitless attempts at transmitting the disease to animals were made with nervous tissue. Since it has recently been shown⁵ that a new member of the pleuropneumonia group of filtrable microorganisms can, while multiplying in certain visceral cells of mice, produce a toxin with a selective affinity for specific parts of the nervous system, it appeared worth while not only to culture the viscera in fatal cases of infectious polyneuritis but also to observe the effect of inoculation of the fresh tissues into various animals. It was in the course of such a study that histologic examination of the human viscera revealed the lesions to be described in the present communication.

REPORT OF CASES

The clinical histories of the three patients whose viscera were examined will be recorded briefly to indicate that they all exhibited the characteristic manifestations of the disease (Table I).

Case 1

J. S., a white male, 40 years old, was well until February 4, 1940 when he developed a "head cold" (sneezing, nasal discharge, sore throat) and complained of "rheumatism" and pain in the lumbar region. Apparently the initial symptoms did not persist long, but on February 13, after a period of relative well-being, he developed headache, malaise and tingling in the hands and feet. The next day he was obliged to go to bed because of weakness of his legs. On February 15 he had no fever but his legs, arms and hands were very weak, and on February 16 he experienced cramplike pains in the thighs and hips and to a lesser degree in the shoulders and arms. On February 17 he still had no fever, but he was weaker, and mucus began to collect in his throat. He was admitted to the hospital on February 18, exhibiting complete flaccid paralysis of the legs and arms, bilateral facial paralysis, dysphagia, and difficulty in respiration. He was put into a respira-

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VISCERAL LESIONS IN INFECTIOUS POLYNEURITIS

(INFECTIOUS NEURONITIS, ACUTE POLYNEURITIS WITH FACIAL
DIPLEGIA, GUILLEIN-BARRÉ SYNDROME, LANDRY'S PARALYSIS)*

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Infectious polyneuritis or neuronitis is the name usually applied to a disease which is characterized by widespread motor and sensory signs pointing to involvement of spinal and cranial nerves (most commonly the seventh) which usually appear after an interval of days or weeks following a mild respiratory infection. An important diagnostic feature is the marked increase in the protein of the cerebrospinal fluid without any change in the number of cells.[†] While the disease has been observed in persons of almost all ages, it has proved fatal, thus far, only in older adults,[‡] and the survivors, as a rule, make excellent recoveries. Although there are some reports¹ which maintain that the nervous system exhibits no pathologic change in this disease, it appears to be fairly well established²⁻⁴ that definite degeneration affecting the axis cylinders and myelin sheaths, with or without inflammatory reaction, is demonstrable in the peripheral nerves, and that in the

* Received for publication November 13, 1940.

† It is our belief, however, that certain patients suffering from this disease may have some pleocytosis.

‡ Since this paper was submitted for publication we have encountered a fatal case of this disease in a boy, 4½ years old, who died in November 1940 with a clinical diagnosis of poliomyelitis. Histological examination of the nervous system revealed no lesions of poliomyelitis, while changes characteristic of infectious polyneuritis were found in the nerve roots.

tor on February 19, but after increasing weakness and restlessness he died on February 21, 8 days after the first appearance of nervous symptoms.

His temperature varied from 100° to 101° F. on February 18, from 100° to 98.8° F. on February 19, from 98.8° to 100.5° F. on February 20, and rose to 102° F. just before death on February 21. The blood Kahn reaction was negative. Two throat cultures were negative for diphtheria bacilli. The cerebrospinal fluid obtained on two occasions was colorless, clear, and contained respectively 100 mg. and 125 mg. of protein per 100 cc., but there was no increase in cells. Only in the last 2 days of life was the pulse disproportionate to the temperature, varying from 104 to 148. The white blood count was 23,000 per cmm., with 97 per cent polymorphonuclear cells.

Case 2

A. M., a white female, 46 years old, engaged in housework, developed a mild upper respiratory infection with nonproductive cough and myalgia on February 15, 1940. She continued to work, however, and felt better in a few days. On March 1 she developed anorexia and nausea severe enough to prevent her from eating, and on March 7 she began to vomit. The vomiting was neither projectile nor related to eating, but was brought on by coughing. On March 8 she first noticed that her legs were weak and felt "dead." On admission to the hospital on March 9 she complained also of numbness and tingling in the arms, and exhibited bilateral facial palsy and flaccid paralysis of the legs and arms. Difficulty in swallowing and in respiration appeared on March 10, and she died in the respirator on the morning of March 11, 3 days after the first appearance of weakness in the extremities.

The temperature was 100.2° F. on admission (March 9), but below 99° F. thereafter. The pulse rate was 80 to 88 on March 9, and, in association with a normal temperature, 118 to 122 on March 10 and 11. The Wassermann reaction was negative on the cerebrospinal fluid. The blood count was normal, and a throat culture was negative for diphtheria bacilli. The cerebrospinal fluid was colorless and clear, and contained 65 mg. sugar, 703 mg. chlorides, and 80 mg. of protein per 100 cc., but no cells were present. The urine contained reducing substance and acetone on admission (the latter perhaps due to starvation), but no albumin. The blood sugar determined on two occasions was 123 and 126 mg. per 100 cc.

Case 3

G. F., a white male, 56 years old, a stationary engineer of a bakelite plant, developed a "chest cold" with a slightly productive cough on February 11, 1939. For the following few days he did not feel well but he continued to work. Between February 14 and February 25 he "never felt better in his life," but on February 27 he suddenly developed pain, weakness and numbness in both legs. By March 2 both the lower and upper extremities were affected, and on March 4 he was admitted to the hospital exhibiting, as predominant symptoms, weakness of all extremities and bilateral facial paralysis. His condition became progressively worse, and he developed paralysis of the eye muscles and of the jaw, and finally respiratory difficulty. He died on March 11, 12 days after the appearance of nervous symptoms.

The temperature was normal until the last day of life when it rose to

TABLE I
Clinical Data on Three Cases of Infectious Polyneuritis with Visceral Lesions

	Age	Color and sex	Primary illness	Initial nervous symptoms	Spinal nerve involvement			Cranial nerve involvement						Cerebro-spinal fluid		Throat culture	Died
					Tendon reflexes	Sensory signs and symptoms	Paralysis	Fifth nerve	Facial palsy	Palatal paralysis	Voice disturbance	Respiratory difficulty	Dysphagia	Cells	Total protein mg. %		
1 J.S.	40	W.M.	"Head cold" and "rheumatism," 2/4/40	Head-ache, tingling in hands and feet, 2/13/40	Arms, o Legs, o	Pain and tenderness; sensory examination not done	Arms, + Legs, +	Bilateral motor weakness	Diplegia	Partial	Hoarse, nasal	Yes	Yes	3 l.	125	No diphtheria bacilli	2/21/40
2 A.M.	46	W.F.	Upper respiratory infection and myalgia, 2/15/40	Nausea, 3/1/40; vomiting, 3/7/40; weakness and numbness of legs, 3/8/40	Arms, o Legs, o	Pain, tenderness, paresis; impaired touch, vibration and position	Arms, + Legs, +	Tenderness of jaws; bilateral motor weakness	Diplegia	Complete	Hoarse, nasal	Yes	Yes	o	80	No diphtheria bacilli	3/11/40
3 G.F.	56	W.F.	"Chest cold," 2/11/39 to 2/14/39	Pain, numbness, weakness both legs, 2/27/39	Arms, o Legs, o	Pain, numbness, tenderness; impaired vibration	Arms, + Legs, +	Hypalgnesia, left; bilateral motor weakness	Diplegia	?	Nasal	Yes	Yes	o 4 l.	130 75	Not done	3/11/39

tial infiltration with various inflammatory cells. Interstitial infiltration with mononuclear cells was also found in the intervertebral ganglia as well as in the gasserian ganglia (Fig. 7) which were studied in case 1. Certain other observations have been made, however, which have not been recorded hitherto. For example, reference is often made to the chromatolysis which can be seen in the nerve cells of the anterior horn of the spinal cord and in certain nuclei of the medulla, and which has been interpreted by some observers as merely postmortem change. Sections of tissue obtained from case 1 soon after death and stained with eosin and methylene blue after fixation in Zenker's fluid with 5 per cent acetic acid revealed a rather striking "zonal chromatolysis" (Figs. 1 and 2). The Nissl substance appeared well preserved in most of the cell with the exception of a distinctly circumscribed zone of varying size which at first suggested an acidophilic, cytoplasmic inclusion but on closer observation could be identified as an area devoid of Nissl substance. In Nissl preparations these zones appeared as vacuoles and were seen in sections from all three cases.

Examination of a number of sections from both olfactory bulbs of case 1 revealed a vessel in the glomerular layer of one of the bulbs with typical cuffing by mononuclear cells (Fig. 6). This finding was particularly puzzling since no such perivascular infiltration was seen in sections from any other part of the nervous system. Whether or not this observation has any special significance in infectious polyneuritis cannot be decided until more olfactory bulbs have been examined in this disease, but it is of interest to note that even in human poliomyelitis such infiltration has been recorded only twice in a study of serial or semi-serial sections of forty-nine olfactory bulbs.⁶

Another finding, of which the significance for infectious polyneuritis can be determined only by future studies, was present in the abdominal sympathetic ganglia of the celiac plexus in case 1 (Zenker's-acetic fixation and eosin-methylene blue stain). A relatively small number of cells exhibited a peculiar degenerative lesion (Figs. 3-5). The striking change consisted not so much of a progressive vacuolization (vacuolization of occasional sympathetic ganglion cells has been noted⁷ in various unrelated conditions), but of the presence of deep-staining, sharply out-

100° F. and reached 102° F. just before death. The pulse rate was 80 to 90 until March 8, 96 to 120 thereafter and 140 before death. The blood Wassermann reaction was negative and the blood count was normal. The cerebrospinal fluid was colorless and clear on two occasions, and contained respectively 130 and 75 mg. of protein per 100 cc., but there was no increase in cells. Examination of the urine revealed nothing abnormal.

ANIMAL INOCULATIONS AND CULTURES WITH TISSUES OBTAINED FROM CASE 1

Pieces of the liver, spleen, kidneys, adrenals, and of the peripheral portions of the lungs were obtained under aseptic precautions within 2 hours after death. They were streaked on 30 per cent ascitic fluid agar plates, which were examined periodically under the microscope for evidence of development of colonies of the pleuropneumonia group. These cultures were negative. While still fresh, the viscera were pooled, ground with sand, and suspended in physiologic salt solution. After light centrifugation, the following inoculations were made: 0.03 cc. intracerebrally and 1 cc. intra-abdominally into each of 8 *mice*, 18 days old; 0.2 cc. intracerebrally and 2 cc. intra-abdominally into each of 3 *guinea pigs* weighing 250 gm.; 0.5 cc. intracerebrally and 5 cc. intra-abdominally into each of 2 *rabbits*; 2 cc. intracerebrally, 10 cc. intra-abdominally, 0.2 cc. intracutaneously into two areas of the abdominal skin of a *rhesus monkey*. Pieces of the spinal cord and medulla, also obtained under aseptic precautions, were similarly prepared and inoculated in the same manner into the same kinds and numbers of animals. The mice and rabbits remained well during 8 weeks of observation, the monkeys during 11 weeks, and the guinea pigs over a period of 18 weeks. Passage of various tissues from some of the inoculated mice into new mice also gave negative results.*

CHANGES IN THE NERVOUS SYSTEM HITHERTO UNDESCRIBED

Examination of the peripheral nerves in all three cases revealed the changes which have already been recorded by many observers; *i.e.*, degeneration of the axis cylinders and myelin sheaths, proliferation of the cells of the sheath of Schwann, and intersti-

* The viscera and nervous tissue of the boy, 4½ years old, who died of this disease in November 1940 were similarly cultured on 30 per cent ascitic fluid agar with negative results. Rhesus monkeys and mice inoculated with the nervous tissue (medulla or olfactory bulbs) remained well.

Liver. The following types of lesions were observed on microscopic examination (Figs. 13-17): (a) focal cellular infiltration in Glisson's capsule, consisting predominantly of mononuclear elements (case 1, Fig. 13); (b) infiltration of interlobular connective tissue or portal canals with mononuclear and occasionally polymorphonuclear cells (cases 1, 2 and 3, Figs. 14 and 15); (c) focal necrosis involving a few liver cells and infiltration of the site with mononuclear and few polymorphonuclear cells (cases 2 and 3, Figs. 16 and 17); (d) focal fatty degeneration and infiltration (cases 1, 2 and 3, Fig. 16).

Here, it is necessary to point out again that these lesions can be missed unless sections are obtained from more than one region of the same organ, or multiple sections from the same block are examined. The least change was found in case 3, in which multiple sections were required to reveal the described lesions, while in case 1, of four regions examined, two showed no change beyond that mentioned in (d).

Heart. The most severe and extensive lesions (Figs. 18-22) in the heart were found in case 2, and consisted chiefly of: (a) diffuse interstitial infiltration with mononuclear and polymorphonuclear cells (Figs. 18 and 21); (b) areas, suggesting necrosis of isolated muscle fibers, infiltrated by phagocytic cells, *i.e.*, foci of "myophagia" (Figs. 18 and 19); (c) focal phlebitis affecting portions of a coronary vein (Fig. 21), in which the wall was edematous and infiltrated with mononuclear and polymorphonuclear cells (Fig. 22).

Case 3 showed also focal interstitial cellular infiltration (Fig. 20), but of much less extent than that found in case 2, being present in sections from one region of the heart but not in those from another. It is also noteworthy that with the exception of the cellular infiltration, the muscle fibers showed no obvious evidence of degeneration in cases 2 and 3. In case 1, however, one could not be certain that there was no degeneration.

Kidneys. The characteristic lesion (Fig. 23), present most extensively in case 2, consisted of an interstitial infiltration with mononuclear cells especially between the tubules. The adjacent vessels were, as a rule, greatly congested, but the tubules and glomeruli themselves were well preserved. These changes were minimal in case 1, in which only two small foci were found in

lined, acidophilic bodies (black bodies in the photomicrographs) distributed throughout the cytoplasm and especially within the vacuoles. The vacuolization appeared to begin at the periphery of the cell (Fig. 3) and to continue toward the nucleus until most of the cytoplasm had been affected (Fig. 4), leaving a structure which still had the outline of the original cell but showed only vacuoles and many red bodies (Fig. 5). One of us (A. B. S.) has observed similar, though somewhat smaller, acidophilic bodies in nerve cells undergoing necrosis as a result of infection in mice with the virus of equine encephalomyelitis. In addition to the cellular changes just described, there was an occasional rather small focus of infiltration with various types of mononuclear cells (Fig. 8). Such focal, interstitial, cellular infiltration has recently been reported⁷ in the sympathetic ganglia of patients dying from a varied number of infectious diseases.

VISCERAL LESIONS

Adrenals. The most marked changes in the adrenals were found in case 1. Grossly, the left adrenal showed marked degeneration, having the consistency of a fluid-filled bag, while the right adrenal was well preserved. Microscopically, lesions were present in both. In addition to the marked degeneration and hemorrhage at the cortico-medullary junction in the left adrenal, three distinct types of lesions were observed: (a) foci of degenerated cortical cells associated with an infiltration of various mononuclear cells (Fig. 9); (b) infiltration with mononuclear cells along the adrenal nerves (Fig. 10); (c) focal accumulations of lymphocytes and plasma cells occupying what appeared to be dilated lymphatics or the sites of degenerated cortical cells, or both (Fig. 12). It should be noted that these changes were found only because sections were obtained from various parts of the adrenals, and that some sections showed no lesions at all. Case 3, in which only one zone was sectioned, exhibited more interstitial infiltration with mononuclear cells than was seen in case 1, and in addition many foci of vacuolization of cortical cells (Fig. 11). One section in case 2 showed only focal vacuolization. Additional sections from the same block, however, revealed focal cellular infiltration at the cortico-medullary junction, and focal phlebitis of the type found in the heart and lungs of the same case.

ably been responsible for the failure to study the viscera in any detail in the past. We have been able to find but two reports in which the condition of the viscera is mentioned. In his communication on the pathology of the cases studied under the pressure of war conditions in 1917-1918, Bashford² stated: "The only change in the liver was a slight and variable infiltration of round cells in the large and small portal tracts such as is found in many febrile (infective) diseases. The kidneys in all cases showed early, patchy, parenchymatous and glomerular nephritis." In a necropsy report by J. Ganim quoted in McIntyre's⁹ paper on infective neuronitis, there is a description of liver lesions which includes all the types which we observed, and it is also noted that there were degeneration of the heart muscle and vacuolization of the cortical cells in the fascicular zone of the adrenals. Our own studies on the viscera in infectious polyneuritis indicate that the adrenals, the liver, the heart and the kidneys may all be affected and that the pathologic changes correspond in type to those observed in such diseases as diphtheria, scarlet fever and typhoid fever, in which a toxin or toxins elaborated by specific microorganisms are believed to be responsible for the visceral manifestations. Our own interpretation of the lesions in the nervous system of patients with infectious polyneuritis is that the primary attack of the causative agent is on the peripheral nerves, which exhibit the oldest lesions, and that the changes found in the central nervous system represent the usual reaction to injury of the axis cylinders. When the primary attack is on the cell body of the neuron, as in the case of poliomyelitis or related virus diseases, the pathology is altogether different and the visible change in the peripheral nerves is the later manifestation. While the nature of the visceral lesions does not in itself exclude the operation of some unknown virus, the visceral and nervous changes taken together are unlike those which would be expected from the effects of a pantropic virus.

It was stated previously that the present study was undertaken for the purpose of obtaining orienting data concerning the etiology of the disease. No agent has been cultivated from the affected tissues since Wilson² has retracted his work after criticism by Arkwright,¹⁰ nor has the disease been transmitted to animals since Bashford's² early inconclusive attempts. It is our

one of three sections. In case 3, in addition to the scattered inter-tubular infiltration, there was complete fibrosis and obliteration of an isolated glomerulus suggestive of early nephrosclerosis. The pathologic changes in these kidneys correspond exactly to those described by Councilman,⁸ especially in certain patients with diphtheria or scarlet fever.

Lungs. There was an acute bronchitis and lobular pneumonia in all three cases, probably the result of aspiration of foreign material in the last days of life. Case 2, which exhibited focal phlebitis in the heart and adrenals, showed a similar change in one of the pulmonary veins.

Other Organs. The spleen in all cases showed marked engorgement of the pulp with red cells, phagocytosis of erythrocytic elements, diminution in size of the malpighian bodies, and slight infiltration of the reticulum with polymorphonuclear leukocytes. There were no other significant findings with the possible exception of a mild, focal, acute colitis in case 3.

TABLE II
Distribution of Visceral Lesions in Infectious Polyneuritis

Patient	Adrenals	Liver	Heart	Kidneys
J.S. (case 1)	+++ (D., C. In.)	++ (C. In., D.)	- ?	++ (C. In.)
A.M. (case 2)	++ (C. In., D., Phleb.)	+++ (C. In., D., N.)	+++ (C. In., N., Phleb.)	++ (C. In.)
G.F. (case 3)	++ (C. In., D.)	++ (C. In., D., N.)	++ (C. In.)	++ (C. In.)

D. = degeneration; C. In. = cellular infiltration; N. = focal necrosis; Phleb. = phlebitis

A summary of the lesions found in the adrenals, liver, heart and kidneys (Table II) shows that, with the possible exception of the heart, each of these organs was affected in all three cases.* A search was made for inclusion bodies but none was found.

DISCUSSION

Acute infectious polyneuritis has been regarded as predominantly a disease of the nervous system, which it manifestly is from a clinical point of view. This viewpoint, however, has prob-

* We have observed similar visceral lesions in two additional cases which came to necropsy since this paper was submitted for publication.

SUMMARY

Pathologic changes are reported in the viscera of three typical cases of infectious polyneuritis. The lesions in the adrenals consisted in the main of focal degeneration and infiltration with mononuclear cells; those in the liver of focal cellular infiltration in the capsule and portal spaces, focal necrosis of liver cells with cellular infiltration, and of focal fatty degeneration; those in the kidneys of focal intertubular infiltration with mononuclear cells, and those in the heart of interstitial infiltration with mononuclear and polymorphonuclear cells and in one case of necrosis of isolated muscle fibers and focal phlebitis. Focal phlebitis was also encountered in the adrenals and lungs of the same case. "Zonal" chromatolysis is described in the nerve cells of the spinal cord and medulla and a degenerative change consisting of vacuolization and the appearance of many acidophilic, sharply outlined bodies in the cytoplasm of some of the nerve cells in the abdominal sympathetic ganglia. Perivascular cuffing with round cells of a vessel in the glomerular layer of one of the olfactory bulbs was observed in the one case in which they were studied.

Mice, guinea pigs, rabbits, and rhesus monkeys were inoculated with a pool of the lungs, liver, spleen, adrenals and kidneys, and also with the spinal cord and medulla from one of the cases, with negative results. Cultures of these tissues for microorganisms of the pleuropneumonia group were also negative. The belief is expressed that the existing data fit best the hypothesis that infectious polyneuritis is caused by a toxin or toxins with affinities for the peripheral nerves and the viscera and elaborated by the microorganisms responsible for the infection of the respiratory tract which usually precedes the onset of nervous symptoms.

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belief that the working hypothesis that may perhaps be pursued most profitably for the present is that infectious polyneuritis is caused by a toxin or toxins elaborated by the microorganisms which are responsible for the infection of the respiratory tract which usually precedes the onset of the nervous symptoms. To this end every attempt should be made to ascertain the bacterial flora in the respiratory tract in patients with this disease, and whether or not toxins which might reproduce the syndrome of infective polyneuritis are elaborated by those bacteria. It should be mentioned here that Bradford² originally reported that diphtheria bacilli were never found in any case of his series, nor have any been found by other investigators, including ourselves, since then. The interval elapsing between the original infection and the appearance of nervous symptoms is similar not only to that observed in the case of certain bacterial toxins, such as diphtheria, but also to that seen in the case of nonbacterial toxins such as are encountered in "Jamaica Ginger" poisoning in which the interval between drinking and the onset of symptoms is 7 to 14 days.¹¹

The realization that infectious polyneuritis is caused by an agent or agents which can attack the viscera as well as the peripheral nerves leads one to inquire about the frequency with which the visceral lesions may be reflected in abnormal clinical signs or laboratory findings. Bradford,² for example, mentioned tachycardia of 100 to 160 with normal temperature and the patients lying quietly in bed; he also noted the presence of small quantities of albumin in the urine in the absence of pyrexia or catheterization and with no evidence of nephritis. It is noteworthy that in our own cases the heart rate was normal or proportional to the temperature except in the last 2 days of life, when the disproportionate increase in the rate might have been caused by nervous as well as by cardiac disorders, and that the urine did not contain albumin. It is not improbable, however, that the severity with which various organs are attacked may vary in different patients, and reference may be made to the case reported by McIntyre⁹ in which the cause of death appeared to be on a cardiac rather than a nervous basis. However, more clinical and laboratory evidence of visceral involvement may follow the knowledge of existing pathologic changes in the internal organs.

DESCRIPTION OF PLATES

PLATE 85

FIG. 1. Anterior horn cells of spinal cord (case 1) showing "zonal chromatolysis." $\times 160$.

FIG. 2. Anterior horn cell of spinal cord (case 1) showing "zonal chromatolysis." $\times 670$.

FIGS. 3, 4, and 5. Nerve cells in abdominal sympathetic ganglia (case 1) showing various stages of degeneration. Note the dark, sharply outlined bodies (acidophilic) in the vacuolated cytoplasm. The lower cell in Figure 5 is an unaffected nerve cell in the same field for comparison. $\times 1030$.

All photomicrographs were made by Joseph B. Homan.

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PLATE 86

FIG. 6. Perivascular cuffing in glomerular layer of olfactory bulb (case 1).
× 160.

FIG. 7. Interstitial infiltration with mononuclear cells in gasserian ganglion
(case 1). × 500.

FIG. 8. Focal cellular infiltration in abdominal sympathetic ganglion (case
1). × 670.

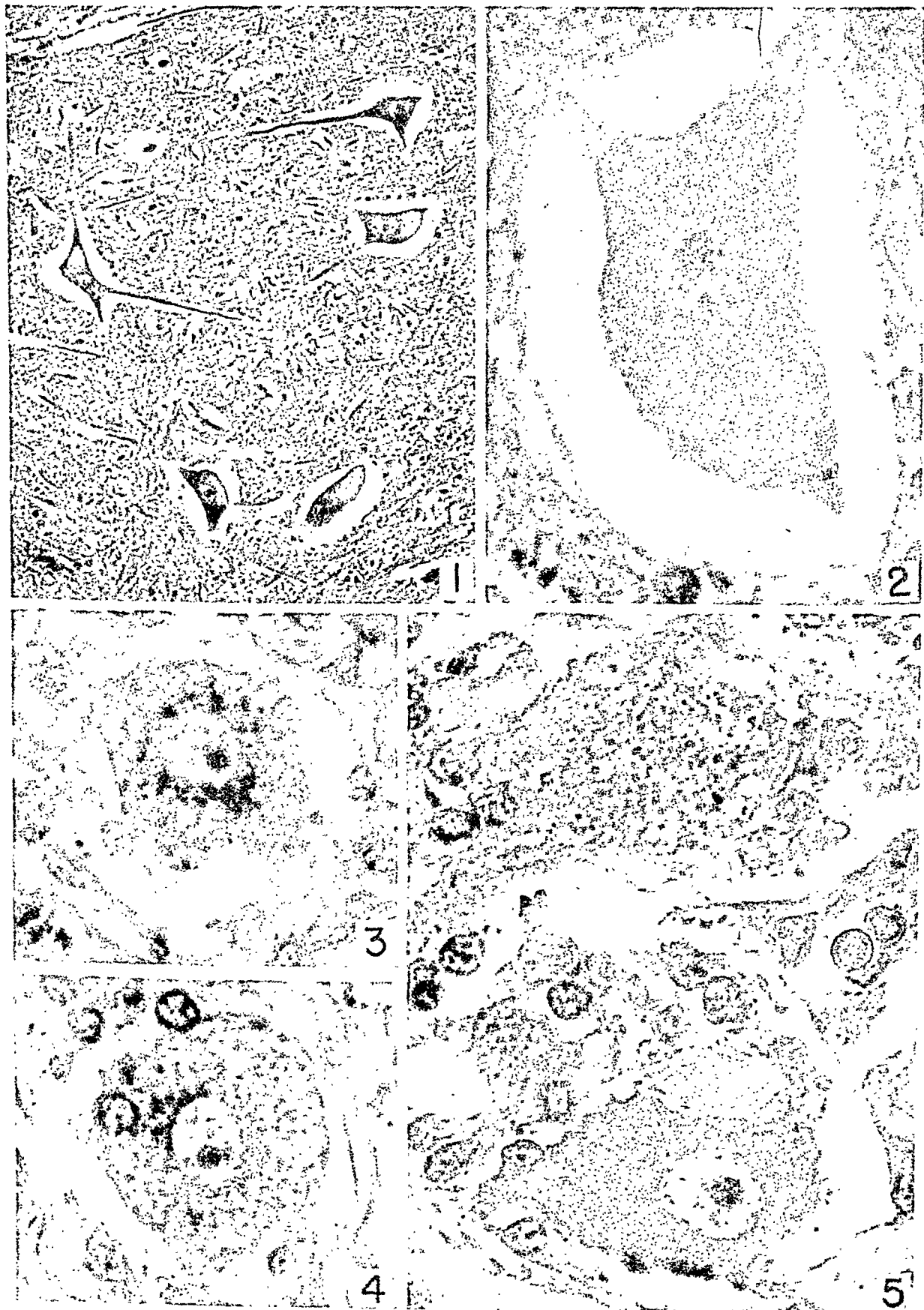


PLATE 87

FIG. 9. Adrenal (case 1); focal degeneration and cellular infiltration. $\times 160$.

FIG. 10. Adrenal (case 1); cellular infiltration along nerve. $\times 140$.

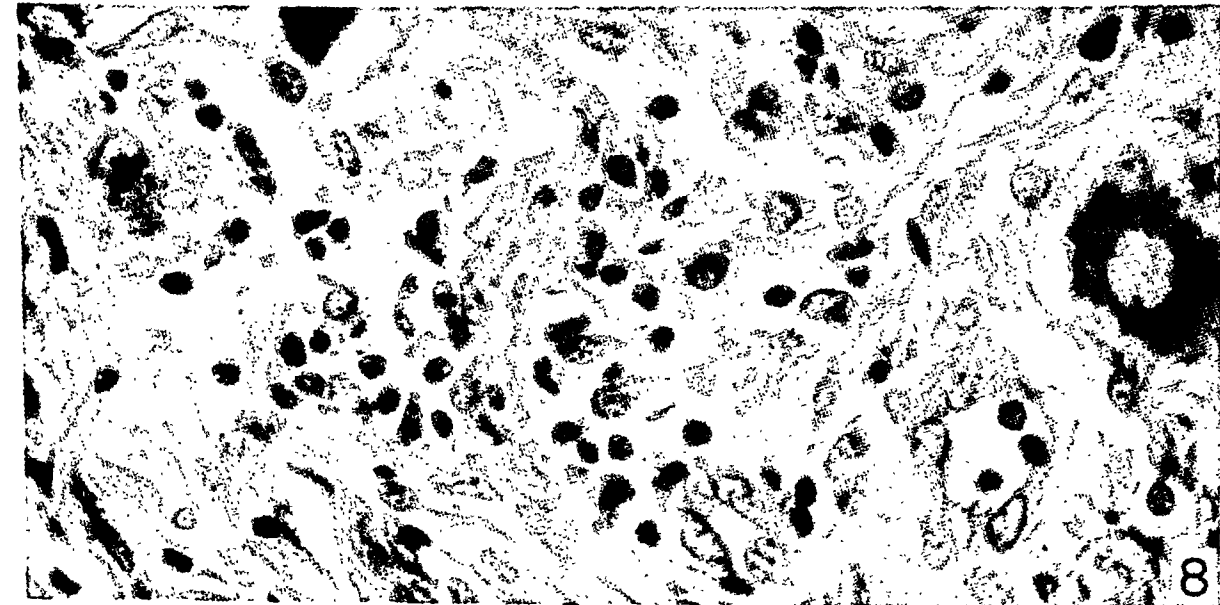
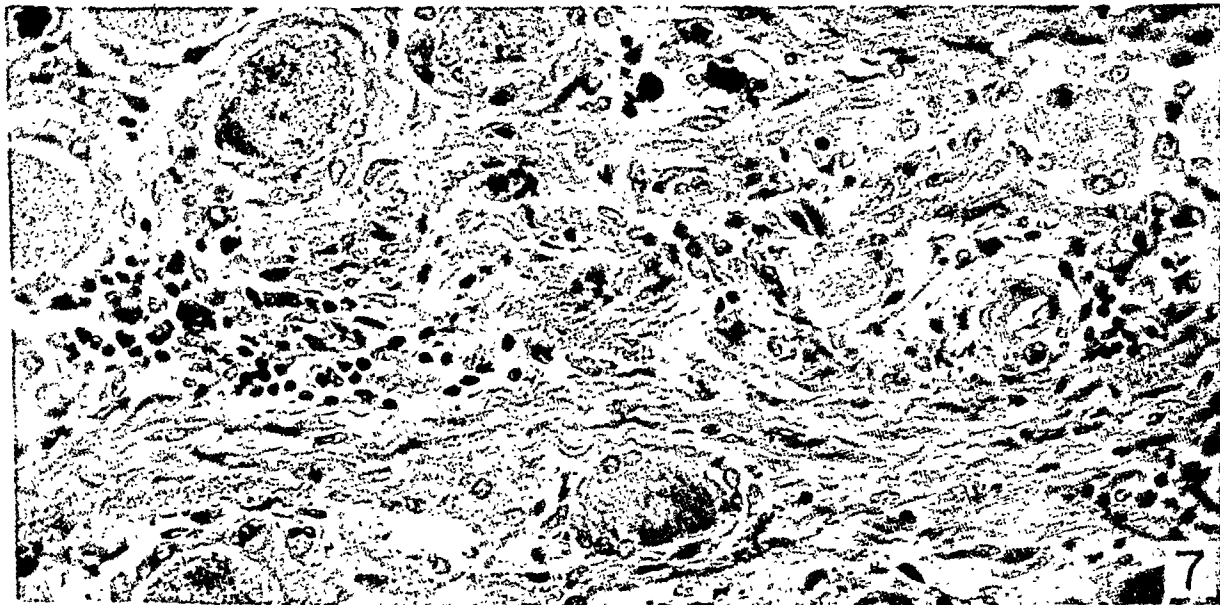
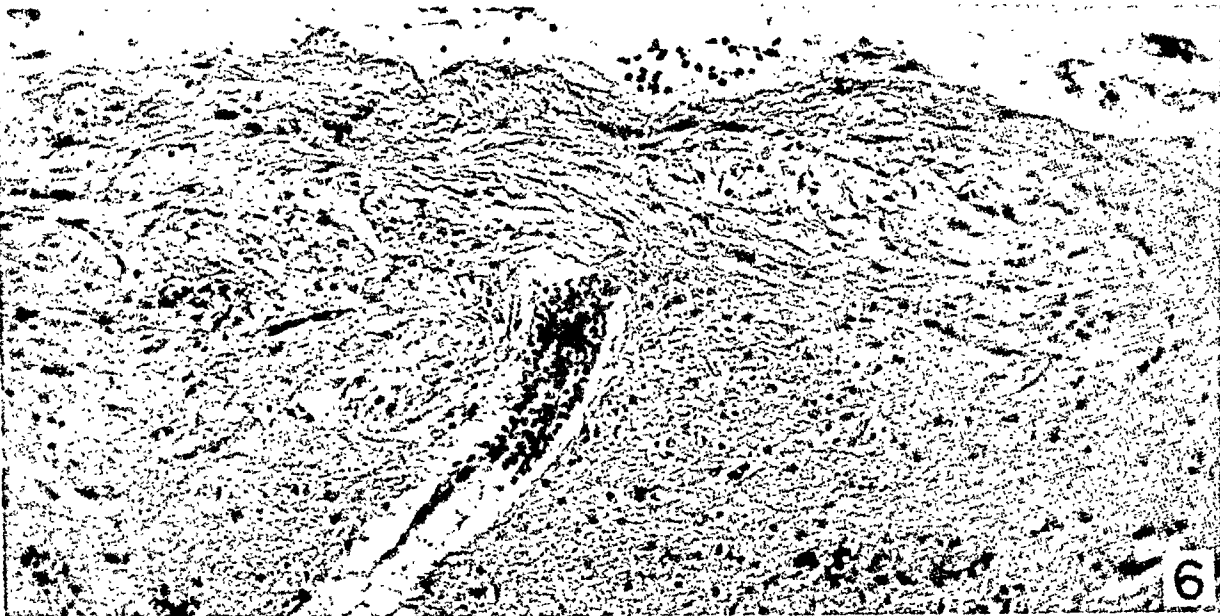


PLATE 88

FIG. 11. Adrenal (case 3); interstitial cellular infiltration and vacuolization of some of the cortical cells. $\times 160$.

FIG. 12. Adrenal (case 1); focal cellular infiltration. $\times 670$.

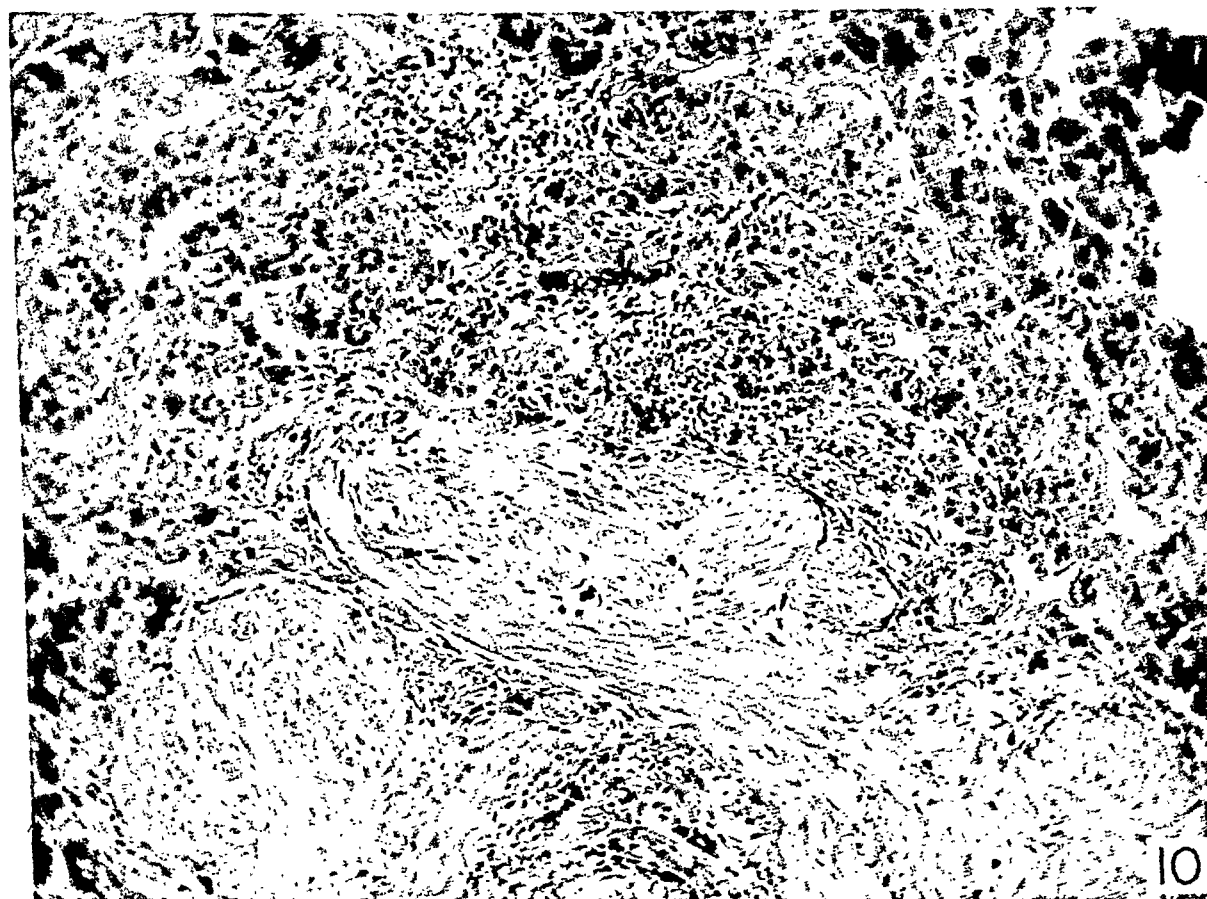
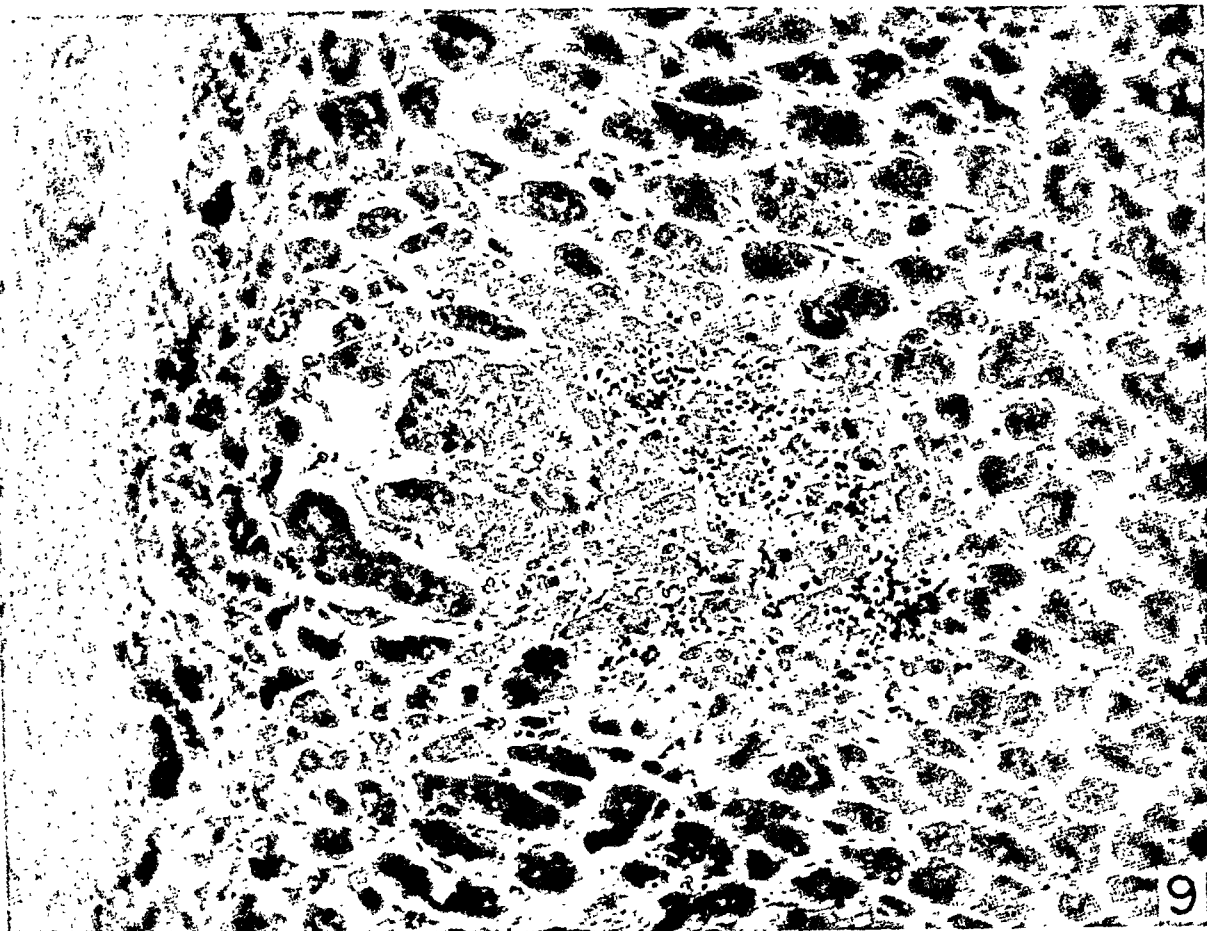


PLATE 89

FIG. 13. Liver (case 1); cellular infiltration in Glisson's capsule. $\times 325$.

FIG. 14. Liver (case 1); cellular infiltration in interlobular connective tissue.
 $\times 160$.

FIG. 15. Liver (case 2); cellular infiltration in portal space. $\times 160$.

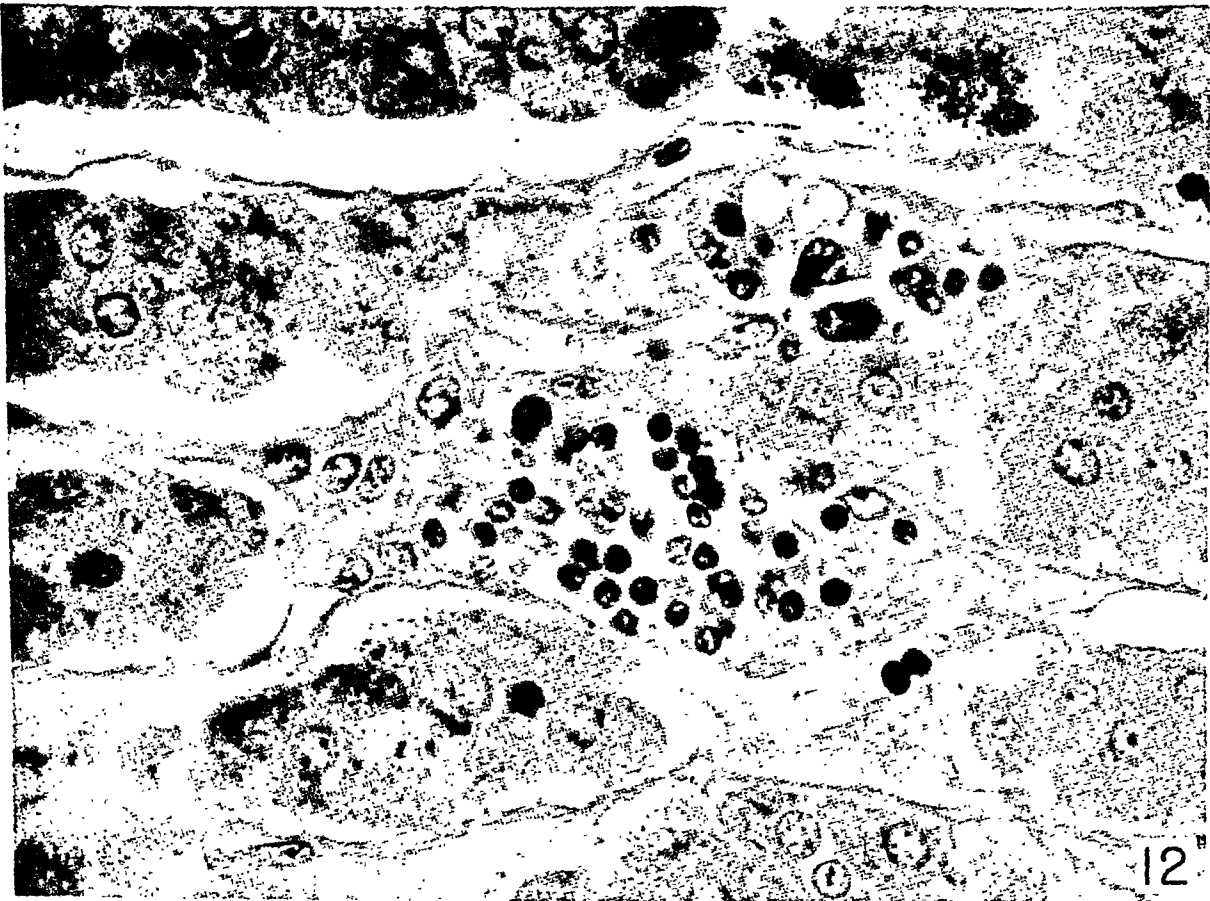
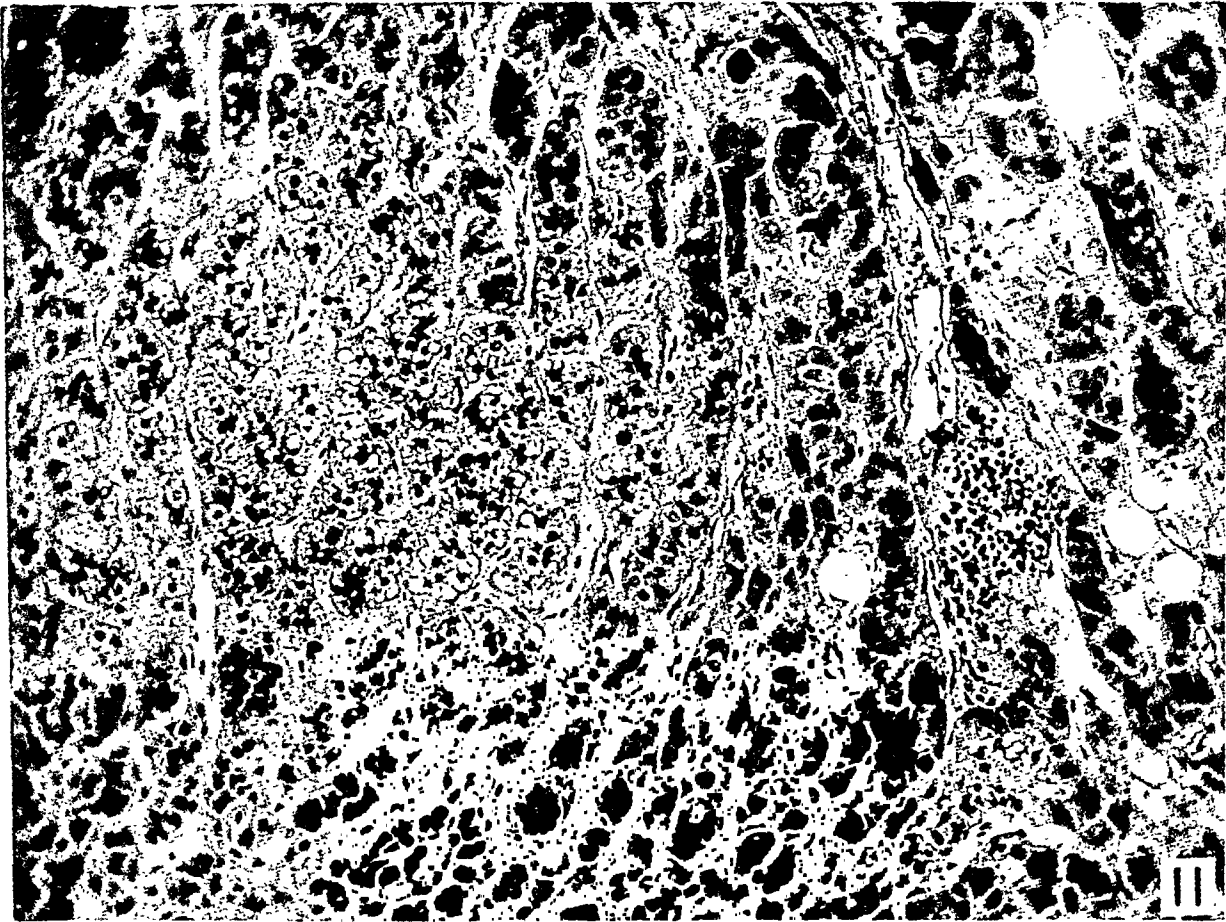


PLATE 90

FIG. 16. Liver (case 2); focal necrosis and cellular infiltration at left and fatty degeneration and infiltration at right. \times 160.

FIG. 17. Liver (case 2); focal necrosis and cellular infiltration. \times 670.

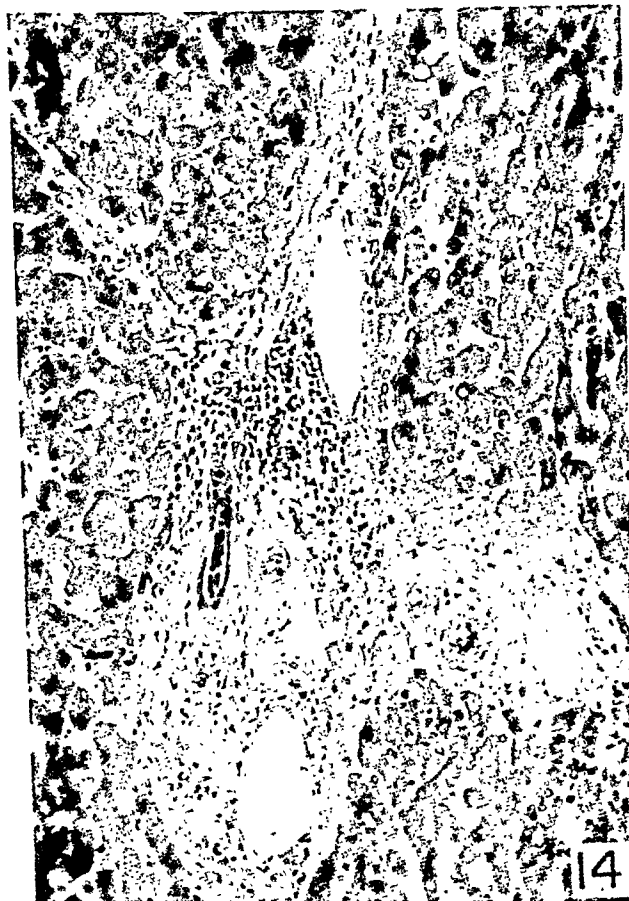
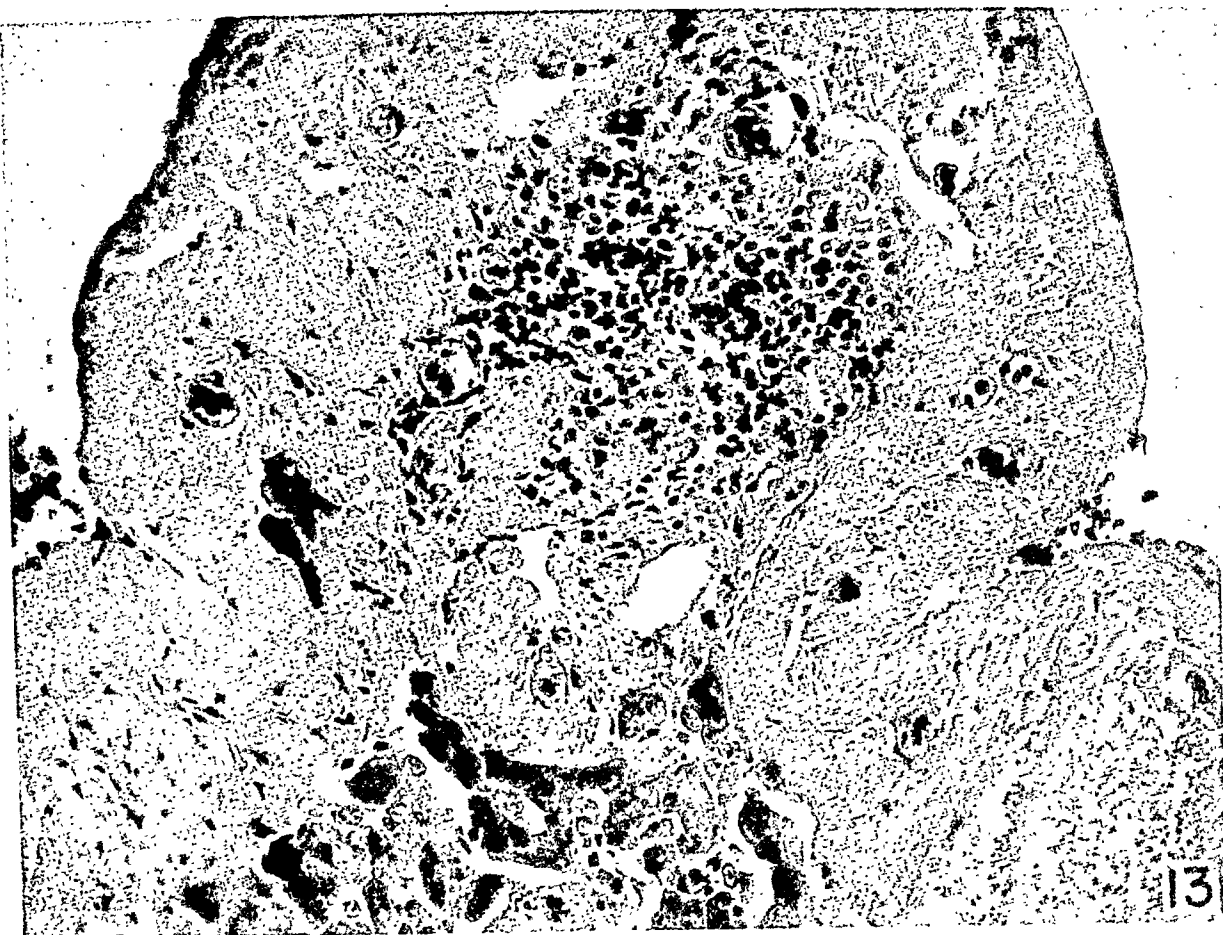


PLATE 91

FIG. 18. Heart (case 2); interstitial cellular infiltration and phagocytic cells surrounding disintegrating muscle fiber at top. $\times 160$.

FIG. 19. Heart (case 2); "myophagia"—phagocytic cells and disintegrating muscle fiber. $\times 1030$.

FIG. 20. Heart (case 3); focal cellular infiltration. $\times 670$.

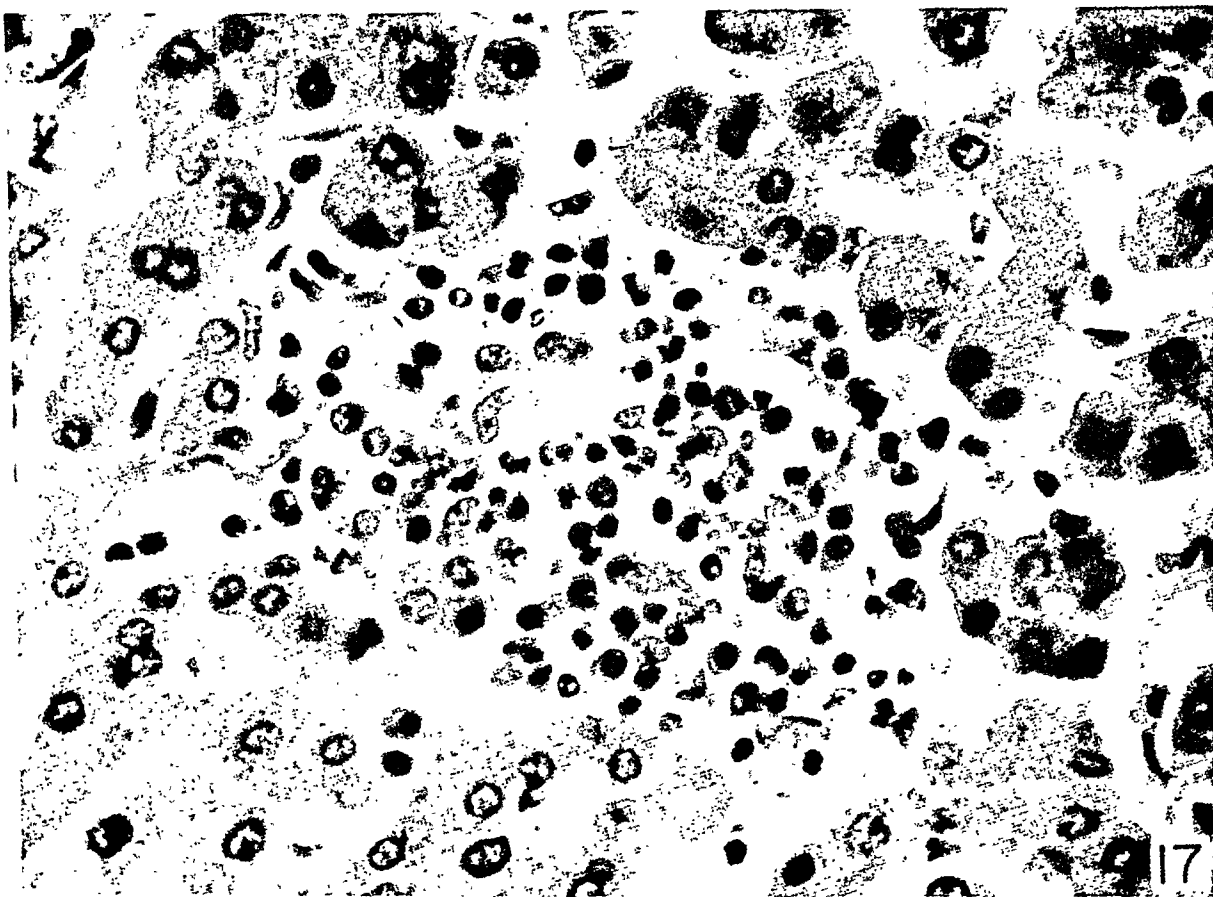
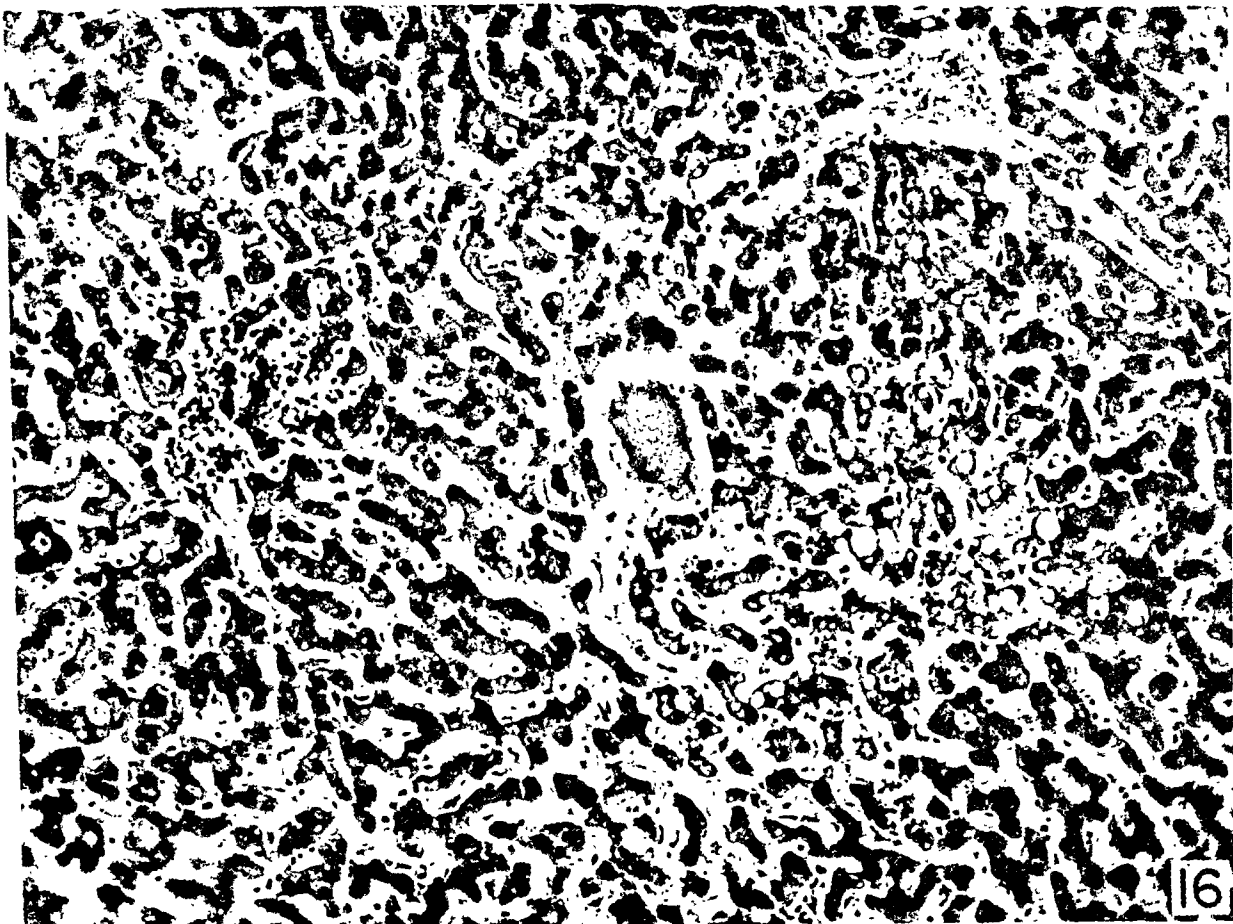
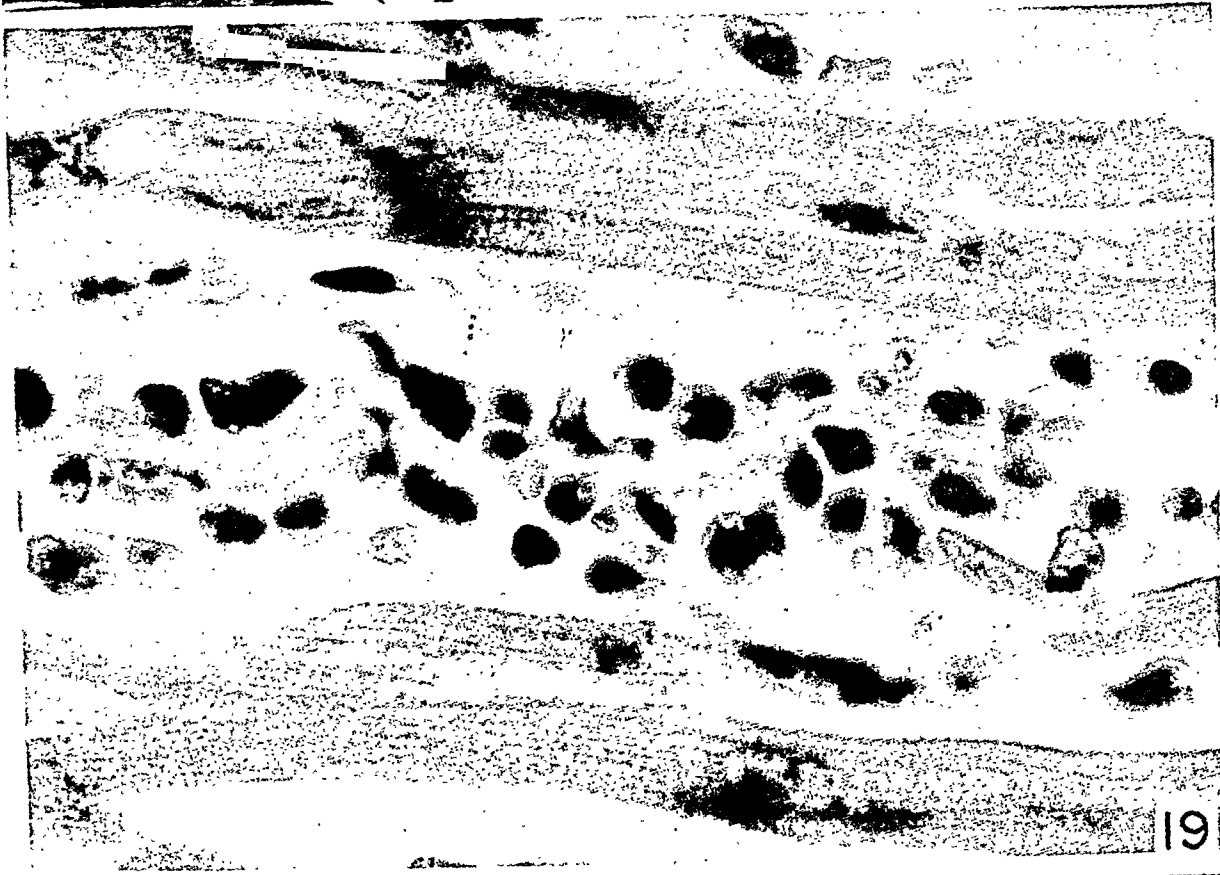
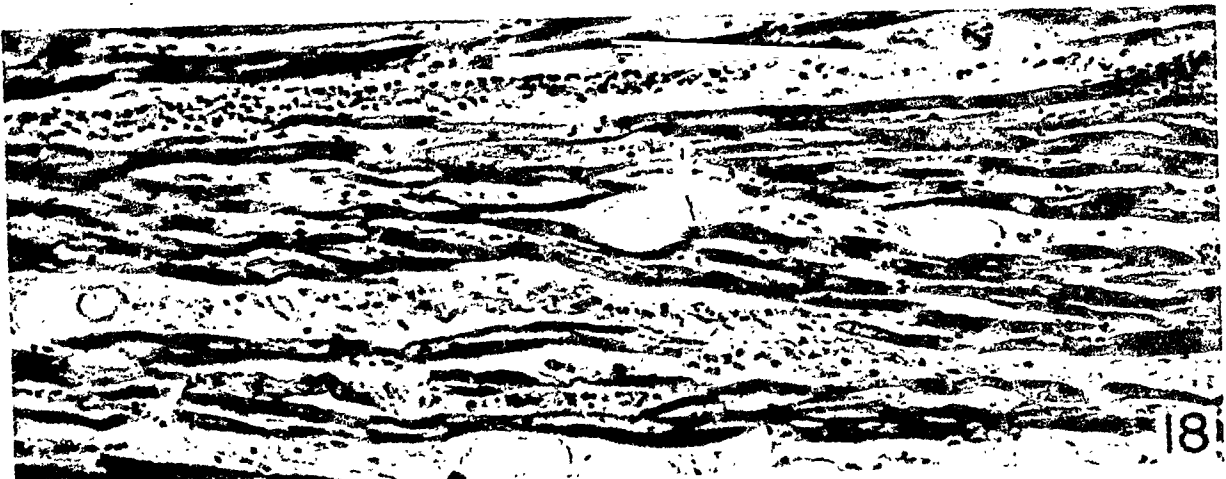


PLATE 92

FIG. 21. Heart (case 2); focal phlebitis and diffuse interstitial cellular infiltration. $\times 57$.

FIG. 22. Heart (case 2); high power detail of wall of coronary vein showing edema and infiltration with polymorphonuclear and mononuclear cells. $\times 670$.

FIG. 23. Kidney (case 2); intertubular cellular infiltration and congestion of adjacent vessel. $\times 160$.



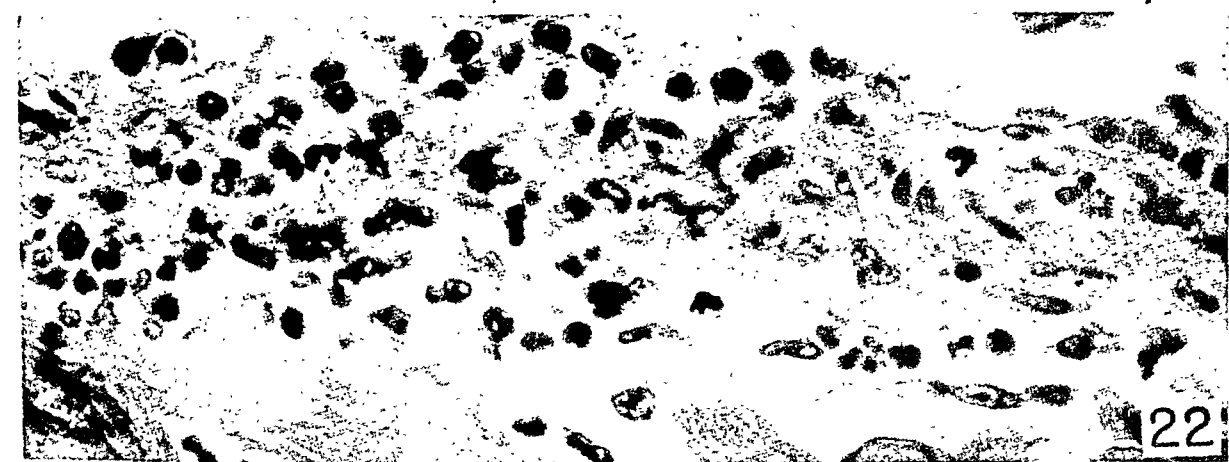


TABLE I
Sixteen Cases of Metastatic Myocardial Neoplasia

Case No.	Type of tumor	Part of heart invaded	Route of invasion	Other lesions of heart	Other metastases
28:131 M., 66 yrs.	Rhabdomyosarcoma of kidney	Myocardium of right auricle	Blood-borne	Hypertrophy, fibrosis, slight coronary sclerosis	Adrenal, abdominal lymph nodes, liver, lung, ribs, vertebrae
30:50 F., 45 yrs.	Scirrhous carcinoma of breast	Subepicardial muscle of left ventricle	Blood-borne to epicardium, thence by lymphatics to myocardium	Atrophy, congestion and edema, hydropic degeneration, lipomatosis, slight fibrosis	Lymph nodes, liver, pleura, adrenal, uterus, pancreas, chest wall
30:193 M., 50 yrs.	Reticulo-endothelioma, primary in skin(?)	Atrioventricular groove; left ventricle; aortic valve	Blood-borne	Hypertrophy, diffuse fibrosis, lipomatosis, coronary sclerosis, edema	Lung, lymph nodes, pancreas, bladder, peritoneum, pelvic connective tissue, skin, voluntary muscle
30:194 F., 75 yrs.	Small spindle cell sarcoma of lung	Pericardium; muscle of left auricle	Direct extension	Coronary sclerosis, atrophy, fibrosis, dilatation	Bronchial nodes, by extension only
30:211 M., 29 yrs.	Melanosarcoma from mole on leg	All parts: all layers	Blood-borne	Diffuse fibrosis	Practically all organs except ureters
31:31 F., 49 yrs.	Carcinoma of bronchus, columnar-celled in some parts, squamous in others	Right ventricle (epicardium, muscle, and endocardium)	Blood-borne(?) to epicardium, thence by lymphatics to myocardium	Coronary sclerosis, edema, hypertrophy, perivascular fibrosis	Opposite lung, bronchial lymph nodes, liver, peritoneum, adrenals, uterus, ovary, mesentery
32:34 M., 57 yrs.	Reserve (or indifferent) cell carcinoma of lung	Right auricle (epicardium and myocardium)	Regional lymphatics (May have been direct extension)	Atrophy, in general; mitral stenosis; hypertrophy of right ventricle; congestion, edema, and round cell infiltration of muscle	Thyroid, pleura, ribs, kidney, lymph nodes
33:145 M., 31 yrs.	Adenocarcinoma of rectum	Right ventricle	Blood-borne to epicardium, thence by lymphatics to myocardium; tumor cells also in blood vessels of myocardium	Auricular thrombus	Pelvis, abdominal lymph nodes, lungs, parietal pleura, spleen, liver

METASTATIC TUMORS OF THE MYOCARDIUM *

A REVIEW OF SIXTEEN CASES

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Metastatic tumors of the myocardium are relatively infrequent. In the autopsy series of the Wisconsin General Hospital, with slightly over 3,000 autopsies recorded at the time of writing, 16 cases had occurred.

Clinically, no sign of involvement of the heart muscle had been found even when extensive invasion had occurred. In every instance, any impairment of cardiac function noted during life was adequately explained by the non-neoplastic cardiac lesions present, such as coronary sclerosis, myocardial fibrosis or valvular lesions.

In 1935 Shelburne¹ reported a primary cardiac tumor diagnosed during life by reason of (1) a comparatively sudden onset of cardiac decompensation without known cause; (2) rapid accumulation of bloody pericardial fluid, which did not clot on standing; (3) lack of positive evidence of tuberculosis or syphilis, and (4) predominance of lymphocytes in the white cells of the pericardial fluid, eliminating the possibility of acute pericarditis. Heart block was also present. Such phenomena were absent in the present series.

Fishberg² recorded 3 cardiac tumors, diagnosed antemortem by the presence of auricular fibrillation in 2, and auricular flutter in the third. The only patient showing fibrillation in the present series (No. 30:194) had noticed an irregular heart beat "as long as she could remember"; little importance therefore can be attached to that phenomenon in this particular case.

Besides the myocardial metastases here reported, there were 23 cases with metastasis to the pericardium, representing 11 different types of primary tumors: carcinoma of the lung, 6 cases; lymphosarcoma, 5 cases; melanosarcoma, 3 cases; carcinoma of the stomach, 2 cases; and endothelioma, carcinoma of the renal pelvis, reticulo-endothelioma, carcinoma of the fundus uteri, car-

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cinoma of the thyroid, perithelial angiosarcoma of the pleura, and Hodgkin's endothelioma, each 1 case.

Table I summarizes the essential features of the 16 cases with myocardial metastases but certain items of special interest may be mentioned at greater length.

In case No. 30:194 a diagnosis of sarcoma of the lung was made. It was recognized that this resembled the tumor usually diagnosed as small cell or oat cell carcinoma, but the growth was massive and apparently metastasized only by direct extension. Further, a reticulum stain revealed reticular fibers in intimate contact with the tumor cells and forming processes which appeared to take their origin from the cells. On this basis the tumor was considered a sarcoma.

Case No. 34:125 illustrated a multiplicity of modes of spread, the bronchi and mediastinum being invaded by direct extension from the primary site in the esophagus, whereas the tumor cells in the myocardium very obviously came by way of the coronary arteries.

In case No. 32:34 there were two primary tumors: a carcinoma of the lung, the major malignancy which gave rise to the myocardial metastasis, and a carcinoma of the prostate which spread only by local extension to the seminal vesicles and bladder. The two tumors differed so widely in their histologic structure that there was no difficulty in determining the source of the metastases.

Case No. 39:41 presented a histologic picture of special interest. The wall of the right auricle was invaded by metastatic cells from a primary tumor in the body of the pancreas, and the right auricular appendage contained a decolorized thrombus. Within this thrombus was a cystic space of microscopic size, lined by cancer cells.

Case No. 36:23 introduced a controversial question, as many authorities doubt the existence of true mesothelioma of the pleura. The diagnosis in this case was made on the following gross and microscopic pathological considerations: the presence of a massive pleural growth which appeared, in the light of careful gross and microscopic scrutiny, to be invading the lung from the pleural surface; the lack of any other site which could be regarded as primary, even after diligent search; and the bizarre morphology of the cells, which in some fields formed sheets suggesting a cov-

Case No.	Type of tumor	Part of heart invaded	Route of invasion	Other lesions of heart	Other metastases
34:125 M., 46 yrs.	Prickle cell carcinoma of esophagus	Myocardium of left ventricle	Blood-borne	Thrombus in cardiac vein, endocardial thrombus, congestion, edema, fibrosis	Bronchi and mediastinum (extension)
36:23 M., 55 yrs.	Mesothelioma of pleura	Scattered throughout myocardium	(1) Blood-borne to myocardium; (2) blood-borne to epicardium, with direct extension to myocardium	Slight hypertrophy, coronary sclerosis, mitral sclerosis and fibrosis, myocardial fibrosis	Lungs, brain, kidneys, lymph nodes, skin, peritoneum, adrenals, pancreas
36:69 F., 57 yrs.	Adenocarcinoma of fundus uteri	Left ventricle, epicardium and muscle (cells in blood vessels)	Blood-borne to epicardium; tumor cells in blood and lymph vessels in myocardium	Congestion, slight fibrosis, cloudy swelling and hydropic degeneration	Lung, lymph nodes, liver, spleen, pancreas, adrenals, kidneys, gall-bladder, peritoneum
36:291 M., 58 yrs.	Squamous cell carcinoma of lung	Left ventricle (epicardium and myocardium)	Blood-borne to epicardium, thence by lymphatics to muscle	Perivascular fibrosis	Bronchial and vertebral lymph nodes and adrenal; chest wall by extension
37:217 M., 69 yrs.	Myogenic sarcoma of bladder	Left ventricle, right auricle, all layers	Blood-borne	Sclerosis of aortic valve, coronary sclerosis, patchy fibrosis, endocardial thrombus	Esophagus, pleura, lungs, liver, spleen, pancreas, adrenals, ureters, colon, abdominal wall
38:111 M., 74 yrs.	Adenocarcinoma of pancreas	Left ventricle, muscle	Blood-borne	Coronary sclerosis, edema and patchy fibrosis	Gallbladder, mesentery, lungs
38:236 M., 45 yrs.	Lymphosarcoma (primary site undetermined)	(Autopsy done elsewhere; incomplete gross description)	Probably blood-borne to epicardium, with direct extension to muscle		Lung, diaphragm, intestine
39:41 M., 69 yrs.	Adenocarcinoma of pancreas	Right auricular appendage	Blood-borne	Auricular thrombus, hypertrophy, perivascular fibrosis	Lungs, lymph nodes, liver, peritoneum, adrenals, spine

it was impossible to determine with certainty the route of metastasis by examination of microscopic sections. For example, in case No. 30:50 the epicardial nodule was so diffuse that it was impossible to ascertain by the microscopic appearance whether the tumor cells were blood-borne or carried through the lymphatics. A review of the gross description, however, revealed that there was an isolated epicardial nodule near the apex of the left ventricle. This fact, added to the presence of metastases in such remote organs as the uterus and adrenal, led to the conclusion that this was a blood-stream invasion of the epicardium.

Again, in a few cases malignant cells were seen in both the blood vessels and lymphatics of the myocardium. Whether the cells in the two vascular systems had been transported independently, or whether this invasion represented the rupture of tumor cells into vessels within the myocardium, could not be determined with certainty.

The modes of growth within the muscle, as distinct from the routes of metastasis, were those characteristic of the various types of tumors and took three general forms: (1) invasion through the channels, such as the lymphatics and blood vessels; (2) infiltrative invasion with varying degrees of myocardial destruction; and (3) massive growth (microscopically speaking), with displacement and complete destruction of muscle fibers.

No primary tumors of the heart were found in the autopsy series from this laboratory.

Consideration of the ages of the patients reveals nothing of particular interest, as in general they correspond to the age groups in which these types of tumors are apt to be found.

Twelve out of the 16 patients were males. This appears to represent a great predominance in the male sex, but such a conclusion is modified when one considers that in 857 cases of malignancy in the autopsy series studied there were 594 males and 263 females, a proportion not far from that found in the series of myocardial metastases.

SUMMARY

Sixteen cases of metastatic tumors of the myocardium are reported, with a tabulation of certain features and a brief discussion. Thirteen different types of primary tumors were repre-

ering tissue and in others were spindle-shaped, resembling cells derived from connective tissue. Undoubtedly many pathologists would call this tumor a carcinoma of the lung and it is impossible to prove that this was not a pulmonary tumor. It was felt, however, that the preponderance of evidence favored the conclusion drawn.

DISCUSSION

An analysis of this series reveals the great variety in type and origin of the primary lesions; in 16 cases, 13 different types of primary tumors are represented. There were 3 examples of carcinoma of the lung and 2 of carcinoma of the pancreas but these were the only types to be repeated. This is in agreement with Yater,³ who found in his exhaustive review of the literature that metastasis to the heart had occurred from neoplasms of all the main organs.

On further examination it appears that 7 of these 16 primary tumors were located in or about the chest. This observation could logically be anticipated, since such tumors are usually in fairly close proximity to the heart. It indicates a possibility of regional lymphatic dissemination which should be borne in mind when the clinical course suggests cardiac metastasis.

The degree of general spread of the tumors in this series is of interest. In 10 of the 16 cases the distribution of metastases could be considered as generalized. On the other hand, in one case (No. 34:125), a carcinoma of the esophagus, the myocardium was the site of the only remote metastasis, the bronchi and mediastinum being invaded by direct extension. Such an occurrence is infrequent. Burke,⁴ for instance, found in his series of 14 cases that the heart was never the sole site of metastasis. Yater³ made no specific observation on this point.

Three routes of invasion, as outlined by both Yater³ and Burke,⁴ are recognized; namely, the blood stream, the lymphatics and direct extension, lymphatic invasion being from the mediastinal lymph nodes against the lymph stream. In the present group, all three modes have occurred. A combination of routes was present in several cases, involving the transport of malignant cells through the blood stream to the epicardium and progression thence into the muscle through the lymphatics or by direct extension. In certain cases in which extensive invasion had occurred,

sented, and there was considerable variation as to route of metastasis and mode of growth within the muscle. In no case had a clinical diagnosis of cardiac invasion been made.

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this period in material submitted from other institutions for consultation. The latter fact testifies to nonrecognition of the fundamental potentialities of the pattern.

It is apparent, however, that this type of mammary cancer, *i.e.*, cancer originating in lobules and terminal ducts, is more common than this incidence would appear to indicate, for, when the tumor infiltrates, it is apt to do so in a peculiar fashion which permits one, after some experience, to recognize the high probability of such origin even though it is impossible actually to trace it. Moreover, in the fully infiltrative form it is often possible to detect outlying areas where lobular carcinomatosis *in situ* is still very apparent. Thus, in these same 300 cases there were 5 in which the pattern was very marked, 2 in which it was moderately developed, and 5 in which it was noted definitely but was scanty in amount.

When this pattern of carcinogenesis is present it is not necessarily the only mode of origin. This may be true, but in some instances a lobular type is combined with different modes of development. Thus there are patterns of combined lobular carcinoma and infiltrating duct cancer beginning in multiple papillary adenomatosis, lobular cancer combined with quite dissimilar large cell comedo-carcinoma both infiltrating and *in situ*, and lobular carcinoma plus tubular adenocarcinoma—again quite dissimilar histologically. Hence it is wholly improbable that this lobular carcinoma *in situ*, or "totilobular carcinoma," as we have occasionally designated it to emphasize its origin in terminal ducts and all constituents of the lobule, constitutes a separate entity other than in the clinical sense and when in its non-infiltrative phase. At that stage involvement of lymph nodes has never been seen.

There is no way in which a clinical diagnosis of lobular carcinoma *in situ* can be made. Patients with cancers of this variety, or with the later infiltrative phase, are in the same age group as are those bearing other mammary cancers. In the noninfiltrative phase the breast reveals none of the classic clinical signs of cancer. The nipple is erect. Retraction is absent. Skin dimpling and fixation are absent. Discharge or bleeding from the nipple has not been noted. The mass is movable and diagnosis is usually "chronic cystic mastitis" or fibro-adenoma. In fact, in our most

LOBULAR CARCINOMA IN SITU *

A RARE FORM OF MAMMARY CANCER

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With increasing emphasis on the early diagnosis of cancer it is obvious that the pathologist will observe increasingly early histologic manifestations of this disease. In recent years it has become apparent to us that various forms of carcinoma *in situ* were being encountered in ever greater frequency and in locations where such phenomena were hitherto but rarely discovered. Examples of entirely noninfiltrative lesions of a definitely cancerous cytology have been accumulated for almost every mucosa-lined structure.

Carcinoma *in situ* in the breast is a disease which has been recognized for many years. The term, however, has not been employed and for the usual form of the disease the designation "noninfiltrative comedo-carcinoma" has served. Nevertheless, comedo-carcinoma constitutes an example *par excellence* of carcinoma *in situ* of a glandular organ. This form, however, is a disease mainly of the larger duct system. One is much less apt to think of carcinoma *in situ* as a disease of small lobular ducts and lobules. The latter process is relatively rare. One of us (F. W. S.) had occasion several months ago to conduct a clinical-pathological symposium† on tumors of the breast at which a lesion of this type was presented. It was found that the malignant character of the process was not recognized by a number of pathologists. For this reason it is felt desirable to review certain features of such tumors.

An impression of the incidence of this type may be gained through a survey of the mammary cancers observed during the past year at the Memorial Hospital. There were two typical examples of strict carcinoma *in situ* of lobules and terminal lobular ducts in approximately 300 primary, operable, mammary cancers. Additional examples of the lesion have been seen during

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† For the American Society of Clinical Pathologists.

is seen. No constant perilobular inflammatory infiltration accompanies the change.

The earliest manifestation of lobular carcinoma *in situ* may be found in isolated cells or groups of cells in the lobule or in the terminal lobular duct (Fig. 3). Presumably multiple isolated cells are the first sign but that stage is soon past. Such cells recall certain features of Paget's disease and we have designated them "pagetoid" cells. The clinical entity, Paget's disease, has not been encountered in this group of cases. In some areas there is a suggestion of general lobular epithelial hypertrophy prior to the stage which one might designate as neoplastic, a graded progressive increase in cell size so gradual that demarcation is impossible. In the earliest phase, the occurrence of pagetoid cells is limited to those cells near the limiting membrane.

It should be emphasized that this lesion occurs in multiple lobules. It has been forcibly impressed upon us that a breast in which this process occurs in the slightest degree constitutes an extreme hazard. Whereas it is not clinical cancer until infiltration occurs, it is always a disease of multiple foci. Hence it is never safe to leave the breast with local excision only, even if the entire palpable lesion has been removed. Whenever the process has been found by local excision, subsequent simple mastectomy has shown additional foci of disease. In our first case, local excision revealed this process and we were unfortunately not aware of its significance. Within the space of a few months the patient had infiltrating cancer with axillary metastases and now has skeletal dissemination. It is our feeling that simple mastectomy is essential, with further procedure dependent on finding the least evidence of infiltration.

The mode of infiltration of these lobular cancers is peculiar and somewhat obscure. One often sees evidence of a sudden, almost explosive liberation of cells from their natural boundaries. The term "explosive" is used with full realization that temporal elements are not known. Nevertheless the resultant picture is often that of a terminal duct, possibly showing the noninfiltrative phase of the tumor, but surrounded by large numbers of isolated, loose cells of rather uniform size but of varying shape (Fig. 4). They are not especially hyperchromatic. In some fields they might readily be confused with large mast cells. In others

marked case the surgeon encountered a gush of fluid on exploration of the mass, a number of small cysts were noted grossly, and cancer remained wholly unsuspected until sections were made. There is no way by which it *can* be recognized grossly. The pathologist sees only congeries of what look like large lobules, if indeed he recognizes anything at all abnormal. Since there is little piling up of epithelium in terminal ducts, necrosis does not occur and hence the chalky streaks so characteristic of many cancers are lacking. Of course, with the development of infiltration the gross morphology becomes that of any mammary cancer. The existence of unrecognizable lesions of this totilobular structure is disquieting to one who trusts his gross diagnosis and suggests the necessity of frozen section in any lesion where disproportion in the size of lobules is evident.

Microscopically the process shows the following characters: There is a sudden and abrupt alteration in lobular cytology (Fig. 1). A group of normal-appearing lobules is interrupted by the presence of a lobule or group of lobules in which, although these lobules may be within normal limits in size or even smaller than normal, the cells are large (Fig. 2). They are perhaps twice the size of those of the normal lobules and their nuclei are in proportion. The nuclei tend to be rather clear; they show no hyperchromatism. The cytoplasm is apt to be opaque, somewhat acidophilic, and occasionally vacuolated. The compact, orderly arrangement of the epithelium of the normal lobule gives place to a decided looseness, a loss of cohesion. Layers do not multiply as layers but cells are progressively displaced toward the lumina in a disorderly fashion, eventually obliterating the space. Slight degenerative changes may result in the formation of central mucoid globules. Mitoses are rare. It is usually necessary to survey a section of two or three entire lobules to find a single mitosis. The cells lose polarity, varying in shape while maintaining surprisingly uniform size. They occasionally assume what looks like a loose reticular structure.

The type of lobule which undergoes this transformation varies. Large lobules, small lobules, lobules with mucoid stroma, metaplastic lobules, and hyalinized lobules, may all assume this pattern. Occasionally only part of a lobule is involved and a sharp line of division between normal epithelium and carcinoma *in situ*

DESCRIPTION OF PLATES

PLATE 93

FIG. 1. Lobular carcinoma *in situ*. At the upper right there is shown a portion of a normal lobule for comparison. $\times 170$.

FIG. 2. Higher magnification of involved lobules in the same area as Figure 1. $\times 450$.

they suggest the morphology of the periductal myoid cells. They are, however, liberated cancer cells and when they metastasize to nodes their form and distribution are such that they might be confused with cells of reticulum cell sarcoma. Their wide infiltration within the breast itself may lead to the invasion of residual lobular connective tissue in lobules which themselves have not given rise to neoplasm. Thus they replace lobules which undergo atrophy. Why atrophy occurs is not known to us. Pressure in this case does not satisfactorily account for it although it may very well do so in the case of expanding mammary cancers of more solid type. The isolated cells may evoke considerable desmoplastic response on the part of the connective tissues.

Since distension of the lobule does not assume marked proportions prior to infiltration, some other factor must be invoked to explain the mass eruption of tumor cells. We suspect some lytic action of the tumor cells, naturally not to be detected by anatomic study.

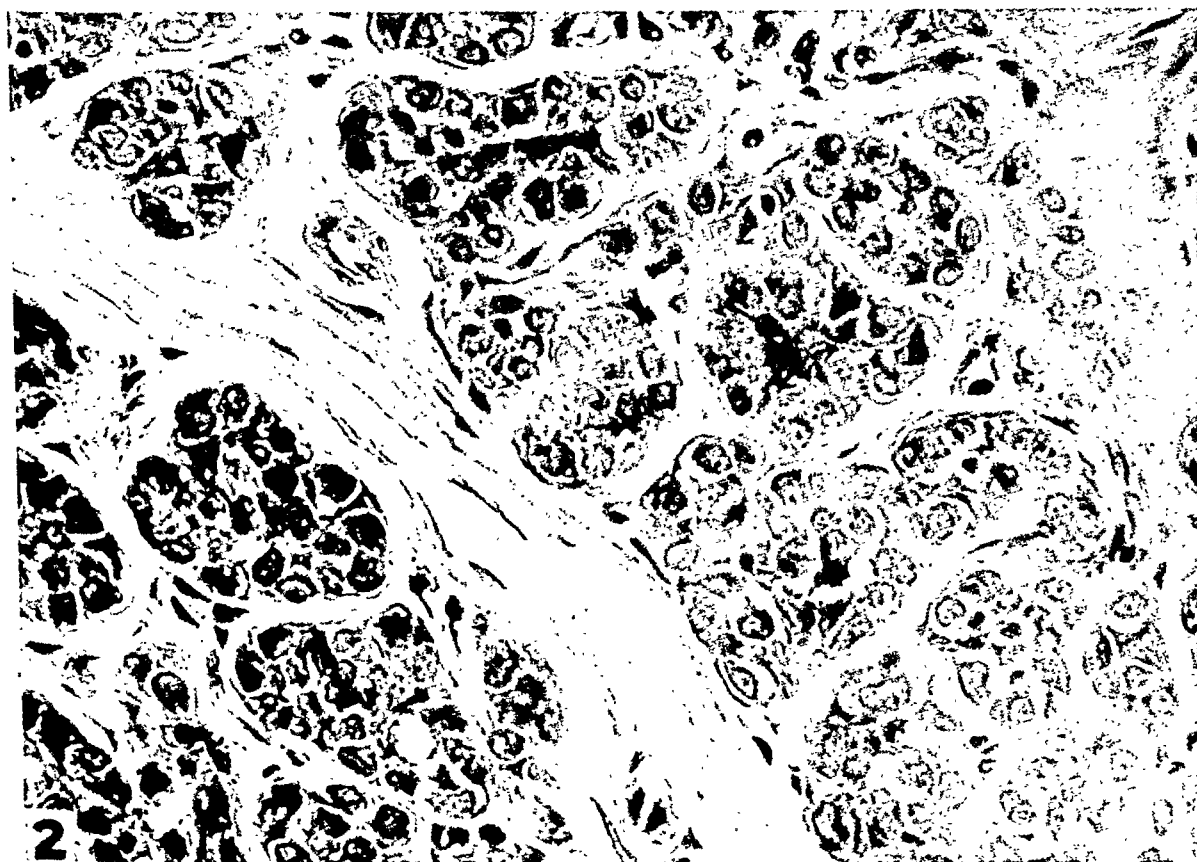
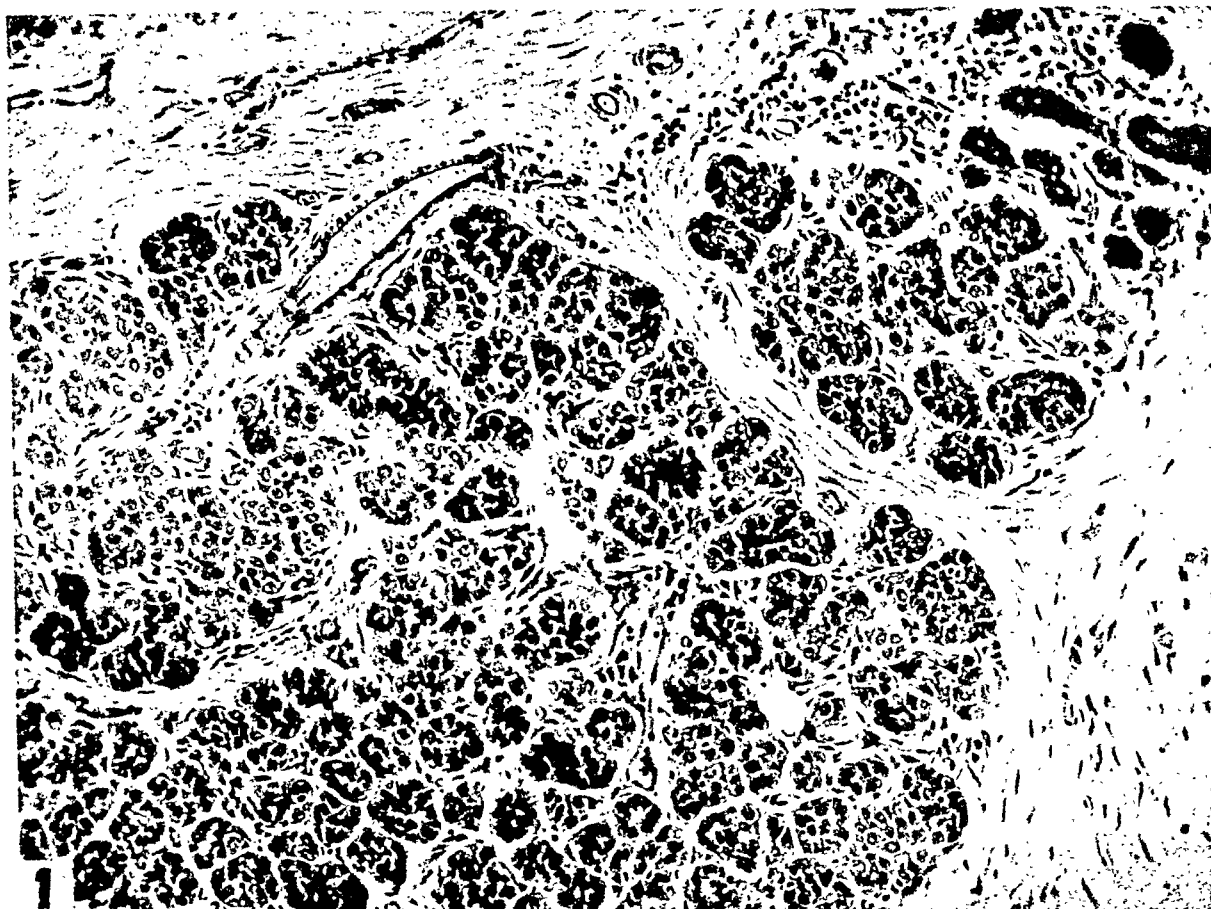
The question has arisen as to whether tumors of this type deserve the designation of "acinar" carcinoma. We have not as yet observed a mammary cancer which in our opinion might properly be considered acinar cancer. It is largely a question of terminology. We do not feel that we can draw sharp lines between terminal ducts and acini. We prefer to regard the acinus as a structure which develops during lactation and which thus constitutes a physiologic phase rather than an anatomic entity. It is to avoid confusion that we employ the term "lobular carcinoma *in situ*."

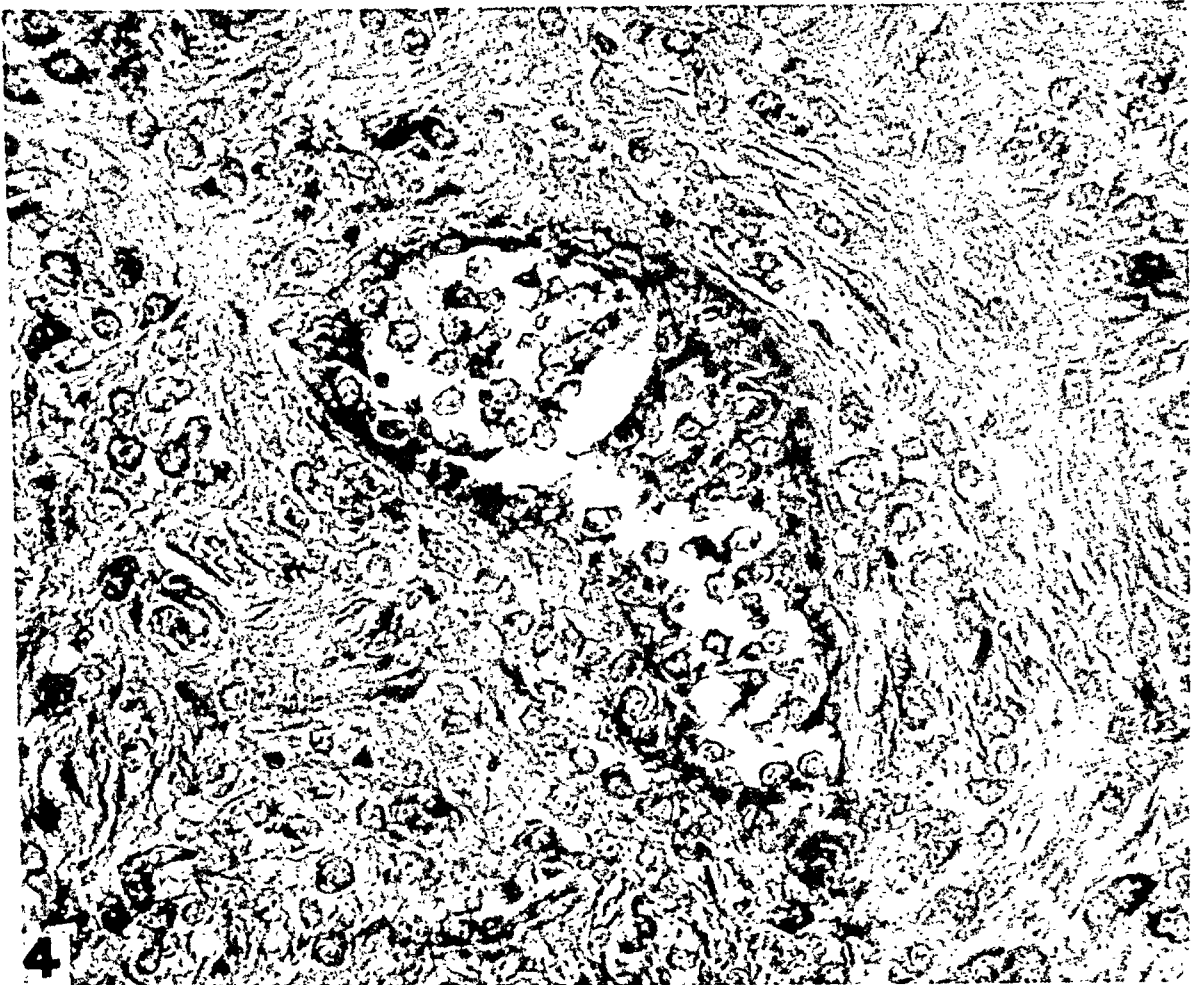
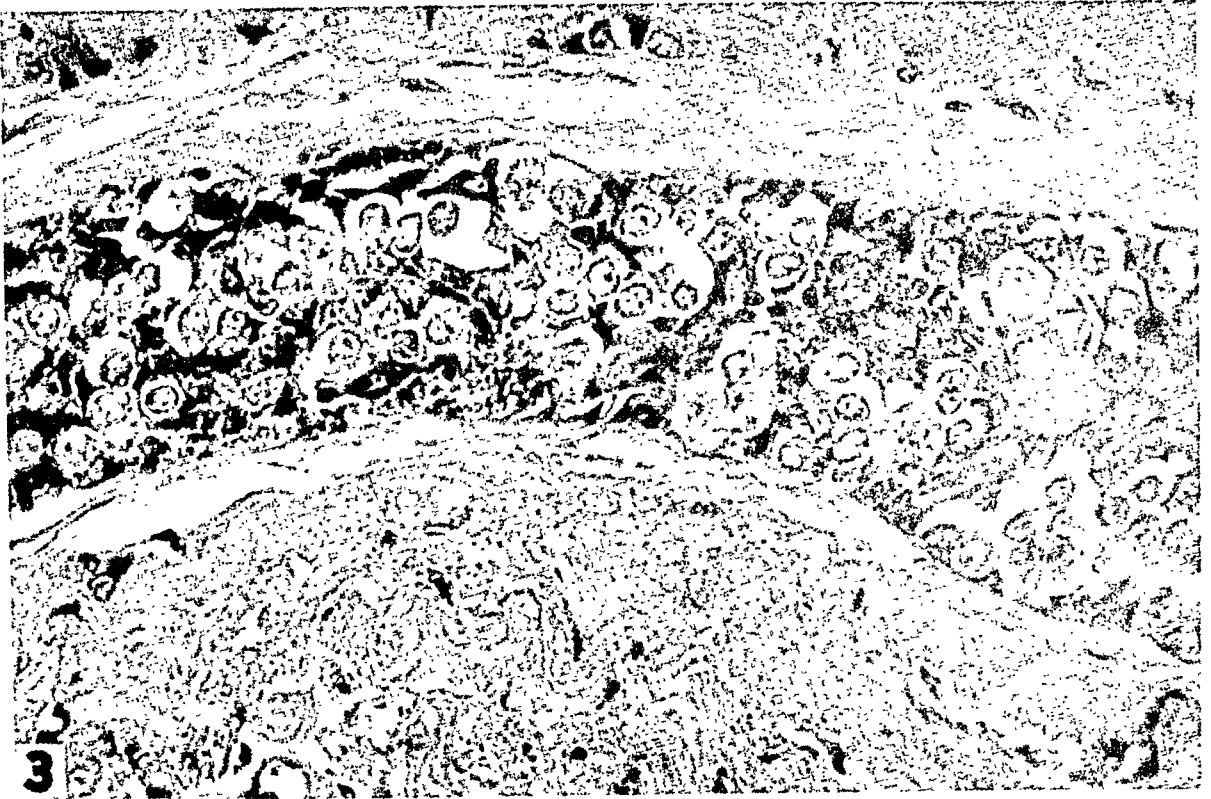
Naturally, the existence of lobular carcinoma *in situ* presupposes that lobules are present. The breast may not be atrophic. Nevertheless there exists a type of carcinoma which apparently takes origin in smaller ducts and which begins in cells of pagetoid type, identical with those seen in lobular carcinoma *in situ*, and this type of cancer may occur in the non-lobule-containing atrophic breast.

PLATE 94

FIG. 3. Terminal duct with "pagetoid" cells. $\times 450$.

FIG. 4. Invasive phase developing from lobular carcinoma *in situ*. A terminal duct with "pagetoid" cells is shown, with surrounding infiltrative cancer cells. Despite the infiltration the periphery of the lobule (not shown) still showed connective tissue encapsulation. $\times 450$.





toms before diagnosis is made and accordingly the process is usually fully developed when recognized. To complicate further the picture from the standpoint of histopathological interpretation, the bulk of material is made unsatisfactory by such factors as scanty biopsy and previous irradiation. Occasionally, however, a particularly suitable specimen is obtained from which certain decisive observations can be made. Recently, we have seen a case which we believe reveals something of the fundamental growth characters of Ewing's tumor.

REPORT OF CASE

Clinical History. The patient, D. D., a boy 15 years of age, was admitted to the Memorial Hospital, Johnstown, Pennsylvania, on May 20, 1940 with the chief complaint of pain in the right chest for 8 years, dyspnea on exertion and pain in the lumbar region for 3 weeks. He gave a history of having had typhoid fever 8 years before, at which time several transfusions were required for epistaxis. Since then he had complained of pain in the right chest, aggravated by exertion. This pain had been intermittent in character. Three months before admission he had an attack of "quinsy" and since had had continuous chest pain. In addition, for 3 weeks prior to admission, there had been pain in the lower lumbar region. Examination of the thorax revealed the left chest to be normal to percussion and auscultation. Expansion was slightly impaired on the right. The percussion note was slightly impaired anteriorly, flat in the axilla and impaired posteriorly from the fifth rib down. Breath sounds were distant or absent in the lower part of the right chest. Vocal resonance was diminished slightly throughout the right lower lobe, but not to the same degree as the breath sounds. Occasional fine, crackling râles were present in the right base posteriorly.

Roentgenologic Examination. Radiographs revealed an irregular ovoid, soft tissue mass which appeared to originate at the right diaphragm and which extended up to the level of the inner end of the right third rib. This mass was rather homogeneous and it was thought that it might contain fluid. Moreover, it was considered to be intrapulmonary rather than in the interlobar fissure. The possibilities of a thick-walled intrapulmonary cyst, a primary diaphragmatic tumor or interlobar abscess were advanced. On the day after admission, a fluoroscopic examination of the chest was done and the mass in the right lower lobe was found to be free from the diaphragm and not to move with diaphragmatic excursion.

An aspiration biopsy was suggested 3 days after admission. Films of the chest showed no change in the primary lesion but at this time scattered, possibly exudative lesions were noted throughout the right lung as well as throughout the lower two thirds of the left lung. At this time attention was called to an involvement of the right seventh, eighth and ninth ribs in the midaxillary line, consisting of what was thought to be a periosteal thickening or periostitis together with a moderate amount of localized bone condensation. This process involved particularly the seventh rib. Two possibilities were considered at this time: a primary tumor originating in the rib and

HISTOGENESIS OF EWING'S TUMOR *

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The origin of Ewing's tumor of bone from vascular endothelium^{1, 2} is a view that has not been uniformly accepted. Connor³ adhered at least in part to the endothelial origin in his first communication on the subject but later⁴ modified this opinion in favor of the reticulo-endothelial derivation, and added that the cell of Ewing's sarcoma might assume osteoblastic properties. Oberling⁵ and later Oberling and Raileanu,⁶ after studying a group of Ewing tumors by special staining methods, concluded that the tumors were derived from a primitive mesenchymal cell, multipotent and capable of differentiation toward endothelium, reticulo-endothelium or into blood-forming elements. Geschickter and Copeland⁷ have suggested that the cell concerned may be the lymphoblast. Roome and Delaney⁸ advanced the possibility that a myeloid stem cell (hemocytoblast) is the active cellular element. In a criticism of possible sources for development of Ewing's tumor, Melnick⁹ asserted that this tumor is neither endothelioma, reticulo-endothelial sarcoma nor myeloma, but is a round cell sarcoma springing from undifferentiated embryonic mesenchymal cells situated in connective tissue about blood vessels in the haversian canals. De Santo¹⁰ has recorded a case which he believed supported the view that Ewing's tumor originated in lymphatic endothelium of the haversian canals and was hence a lymphangio-endothelioma. All of these authors at least accept the existence of a disease commonly called Ewing's tumor of bone and yet Ewing's tumor as an entity has been questioned by Colville and Willis¹¹ and again by Willis.¹²

One of the great difficulties encountered in studying the origin of certain tumors lies in the unfortunate manner in which these tumors occur. Primary growth patterns are frequently obliterated and little or no opportunity for observing their mode of progression remains. In the case of Ewing's tumor, this is eminently true. This group is characterized by relatively long duration of symp-

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tained by the tumor. This is a necessary attribute of any tumor if proof of origin from vascular endothelium is to be obtained, a point that has been stressed by Melnick.⁹ In Figure 2, vasoformative properties are not preserved and the only resemblance to endothelium which remains is the persistent spindle or elliptical shape of some of the cells. Diffuse overgrowth has now occurred and fully developed diffuse endothelioma is pictured. This is the structure most commonly seen in Ewing's tumor with the exception that the individual cells are more spindle-shaped and elliptical. The preponderance of this growth type in these tumors is responsible for Ewing's^{1, 2} term, "diffuse endothelioma."

The occurrence of cells in rosette arrangement is another property often noted in Ewing tumors, though by no means peculiar to this tumor. A group of these structures is seen in Figure 3. They are not numerous in this case. The individual cell type here is that usually encountered in Ewing tumors. Other features present, but not illustrated here, included areas of reactive new-bone formation at the advancing edge of the tumor. The occurrence of this new-bone formation was not seen at every peripheral area. Instead, several areas showed clearly the spread of the tumor into the marrow spaces and haversian canals with no concomitant proliferation of osteoblasts. In the sites of bone formation we were unable to trace transition stages from the tumor cells to osteoblasts. We do not believe that the cells giving rise to Ewing's tumor have any osteoblastic capabilities, inasmuch as bone formation does not occur in the visceral or lymph node metastases of this tumor. Bone formation is reactive and accompanies periosteal elevation.

In the case presented it is difficult to escape certain conclusions. Definite vasoformative properties are shown and the structure seen in Figure 1 is scarcely one that could be duplicated by multiple myeloma, lymphosarcoma, metastatic neuroblastoma or metastatic bronchogenic carcinoma, the tumors commonly mentioned as causing confusion with Ewing's endothelial myeloma of bone. It is not possible to determine whether this particular tumor is derived from lymphatic endothelium or blood-vascular endothelium. Ewing has formerly favored perivascular lymphatic endothelium as the probable site of origin, having traced one of his early cases² to this location. At the present

extending in an intrapulmonary direction, and, because of the history, an interlobar abscess, which, by direct extension, had involved the ribs.

On June 5, a bronchogram with lipiodol was done. This showed an elliptical shadow which seemed to follow the lower interlobar fissure and on the basis of this finding a thoracotomy with biopsy was urged.

Surgical Procedure. Operation was done on June 25. Under cyclopropane anesthesia, a curved incision was made parallel to the right seventh rib, the center being in the midaxillary line. The periosteum of the rib was much thickened. The intercostal nerve was necrotic as a result of either osteomyelitis or tumor. About 3 in. of the seventh rib were removed. On attempting to locate the parietal pleura, it was found that what seemed to be tumor had involved this structure and extended to the periphery of the lung in that region.

Histology

The microscopic diagnosis of the tissue removed at operation was Ewing's endothelial myeloma of bone. The pertinent histological features are illustrated in the photomicrographs appended, all being taken from an area of bone involvement. These fields lay within an area not exceeding 1 cm. in diameter. In Figure 1 is shown what we believe to be the basic pattern of endothelial myeloma. Here, the tumor retains at least a vestige of organoid character, this being represented by formed vascular spaces lined by elongated cells which closely resemble endothelial cells and lie in a relationship expected of this cell. These cells are not entirely uniform, as might be expected. There is some variability in size and shape and some are more heavily stained than others. At one point, enclosed in a vascular channel, is a mitotic figure, poorly shown in the illustration, being slightly out of focus with the remainder of the field. These channels do not contain erythrocytes or leukocytes and hence it cannot be determined whether they are related to lymphatics or to blood vessels. Careful survey of this immediate area fails to reveal the presence of any actively participating cell that might belong to the hemopoietic series, nor do the cells under consideration resemble reticulo-endothelial cells. The proliferative qualities of these cells are readily shown by the gradual coaléscence of groups of single cells to form small masses in which no vascular channels are seen. The structure is more than a little suggestive of areas that may be seen in hemangioma hypertrophicum cutis or in some cellular subcutaneous lymphangiomias. The chief importance of Figure 1 is that it demonstrates clearly the vasoformative properties re-

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12. Willis, R. A. Metastatic neuroblastoma in bone presenting the Ewing syndrome, with a discussion of "Ewing's sarcoma." *Am. J. Path.*, 1940, 16, 317-331.
13. Ewing, James. Personal communication.

DESCRIPTION OF PLATE

PLATE 95

- FIG. 1. Basic, capillary, angiomatous, vasoformative structure of Ewing's tumor. $\times 500$. (The three figures on this plate were photographed from closely adjacent areas of the same neoplasm.)
- FIG. 2. Transition to diffuse endothelioma without total loss of resemblance to endothelial cells. $\times 500$.
- FIG. 3. Cells in rosette arrangement. $\times 500$.

time, he is less restricted in his view and believes that lymphatic or blood-vascular endothelium may be concerned. The ultimate cell type he now designates as "vascular endothelium possessing angioblastic properties" and for some time he has employed the term "capillary angiosarcoma."

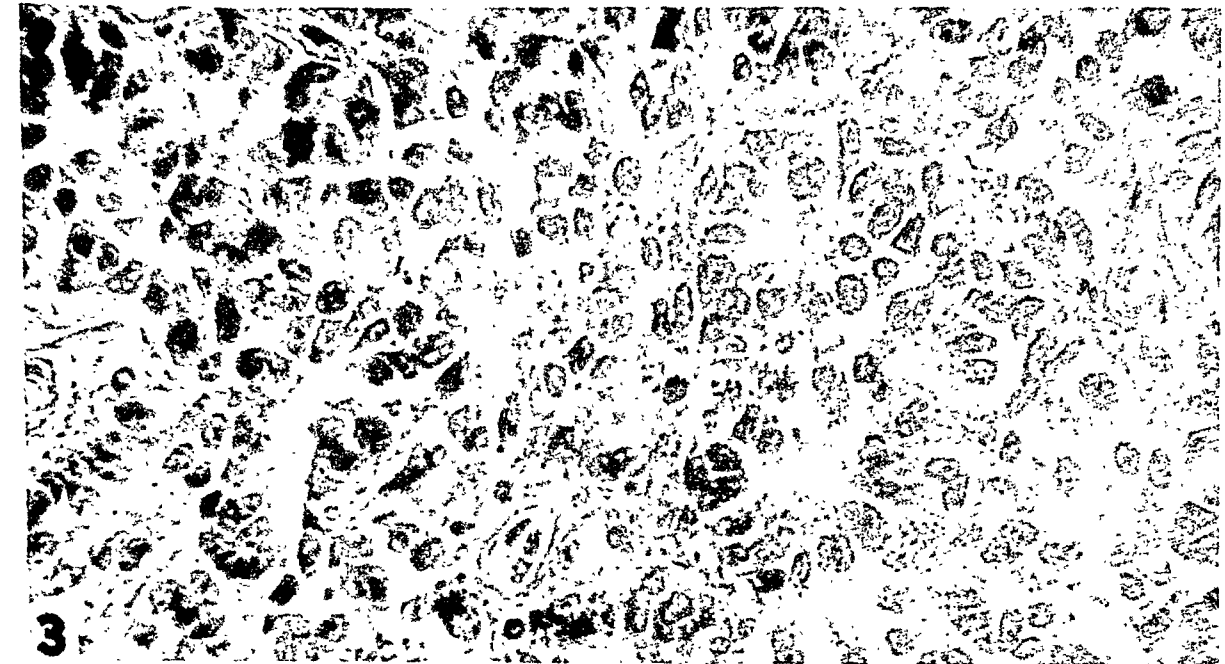
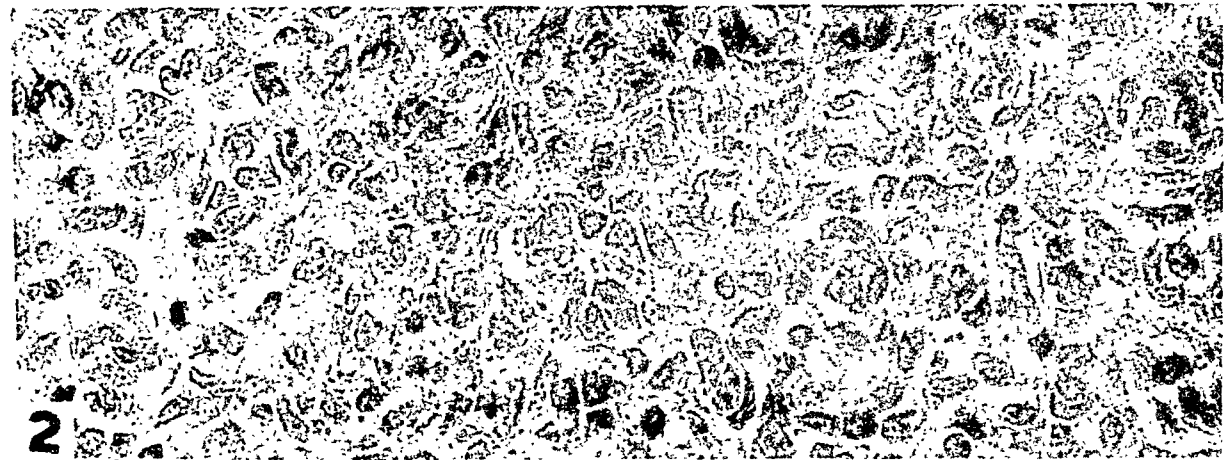
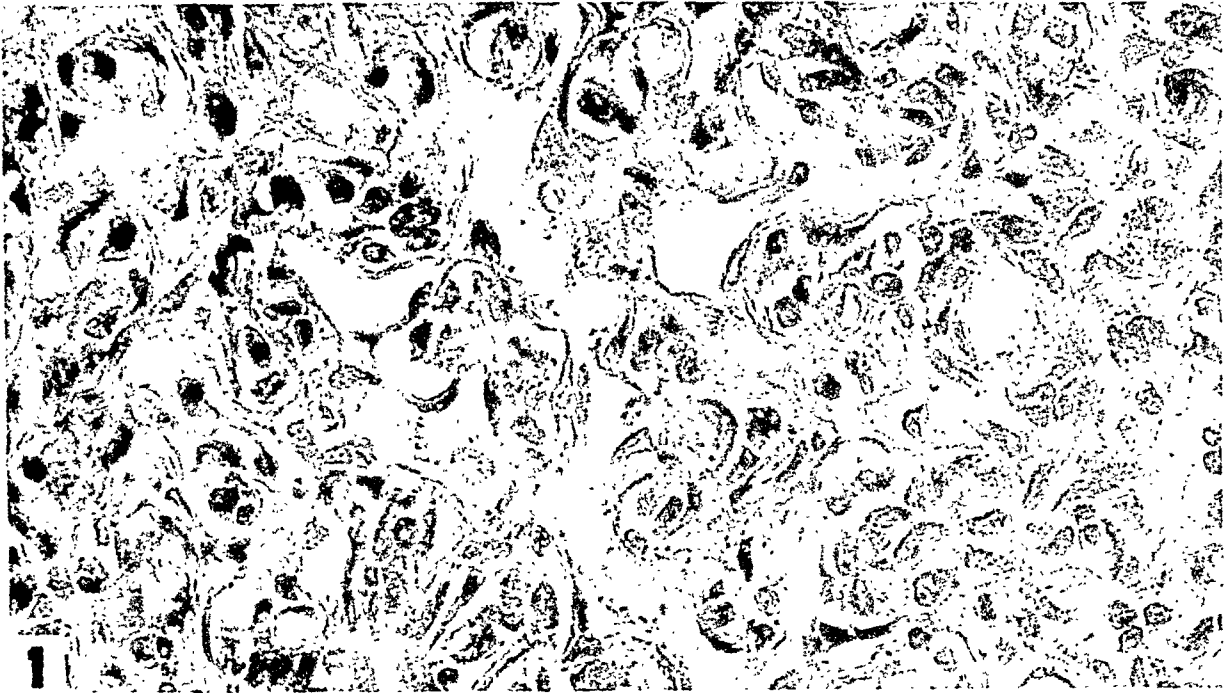
Ewing¹³ has also commented on the obscurity of any etiological factor in endothelial myeloma and has called attention to a possible relationship to chronic osteomyelitis. In the present case, it is of interest to note that for 8 years, following typhoid fever, this patient complained of pain in the right chest, suggesting the possibility that typhoid osteomyelitis may have been present and that an endothelial tumor may have arisen in the granulation tissue.

SUMMARY

A case of Ewing's endothelial myeloma of bone is presented that is clearly traceable to vascular endothelium. The structure shown by this tumor is regarded as wholly inconsistent with anything known in multiple myeloma, reticulo-endothelial sarcoma, lymphosarcoma, metastatic carcinoma or metastatic neuroblastoma. The thesis of origin of Ewing's tumor in vascular endothelium is sustained.

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of bone. As a matter of fact, the "cured" cases of Ewing tumors of bone, all with pathologic material, in the collection of the Registry of Bone Sarcomas of the American College of Surgeons, were recently reviewed by Stewart³ and eleven were accepted as examples of this disease. The longest "cure" was of 31 years.

That metastatic neurocytoma can produce the radiographic picture of Ewing's tumor of bone cannot be doubted. In the Memorial Hospital series this is extremely common in retinocytoma. Almost every case of terminal, highly malignant retinocytoma will yield multiple bone metastases, most commonly subperiosteal and especially near epiphyseal lines, less often medullary. Such metastases are radiographically indistinguishable from Ewing's tumor.

It is true that there are few cases of Ewing tumor of bone with adequate autopsy study. For this reason the following apparently typical case is recorded.

REPORT OF CASE

The patient was a male, 14 years of age. He was admitted to the hospital on March 13, 1940. Two months prior to admission he had sustained an injury while playing. Immediately thereafter a painful swelling of the thigh was noted. Continuous fever developed, the tumor of the thigh continued to increase in size and 3 days prior to admission it broke down and discharged sanguinous fluid.

The patient was wasted, anemic, and very ill. The thigh was enormously swollen. The tumor was globular in shape and measured, by external palpation, 76 cm. in maximum circumference, 43 cm. in length and 28 cm. in thickness. It was tender and a discharging wound was present. The condition was obviously a terminal one. No further investigations were made and the patient died 2 days later of terminal pneumonia.

Gross Findings

Complete autopsy was performed. The primary tumor was situated in the femur. It measured 48 cm. in maximum circumference. The cut surface measured 33 by 18 cm. The major portion of the mass was grayish, cystic and necrotic. A few areas, particularly at the marginal portions of the tumor, were whitish and appeared to be very cellular. Here the tissue was intact. The bone showed two pathologic fractures. The cortical portion of the shaft appeared necrotic and rarefied; the medulla appeared structureless. Save for the fact that the tumor surrounded the femur and had caused double patho-

ENDOTHELIAL MYELOMA (EWING'S TUMOR OF BONE) *

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In 1933 Colville and Willis¹ concluded that the subject of Ewing's tumor of bone was in a chaotic state and that the occurrence of a primary growth of bone of this nature was still unproven. These authors expressed the belief that metastatic tumors of bone of various types, especially neuroblastoma, will probably prove responsible for many cases. As to the case in question the conclusion was reached that the tumor had been primary in the adrenal, although a tumor of the femur appeared to have been extremely extensive, widely disseminated metastases were present in many bones and viscera, and tumor nodules were present in *both* adrenals. In fact, the cut surface of the right adrenal, as illustrated, showed a score of small nodules, both cortical and medullary, the size of the organ was not definitely abnormal, and the pathological picture failed to correspond to that of the commonly recognized adrenal neuroblastoma, since it is usually impossible in fatal disease of this type to be able at autopsy to recognize any adrenal at all. The supposed neuro-epithelial rosettes failed to reveal fibrillary processes with appropriate silver stains.

In a more recent report Willis² reached an essentially similar conclusion, this time despite the fact that the femoral tumor had existed for 3 years. He believed the primary tumor to have been retroperitoneal but failed to show how this mass was to be distinguished from metastases in adjacent lymph nodes. He likewise seemed to accept the neuro-epithelial nature of the rosettes although the only stains capable of offering proof of this contention apparently were not done. Willis asked how rosettes can be characteristic of "endothelial myeloma" when they are commonly present in neuroblastoma, evidently assuming that the term "characteristic" must mean that the feature may never be possessed by more than one thing. He dismissed the 5-year cures of Ewing's tumor of bone as unconvincing and apparently was willing to assign them to confusion with osteomyelitis or syphilis

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ferentiated or syncytial reticulosarcoma. In the second there is some degree of differentiation with formation of reticulin fibrils. The third is frankly dictyocytic and the fourth lymphoblastic. Ewing⁵ has recently expressed himself as follows:

"Oberling and Raileanu⁶ and others have presented evidence to show that this tumor arises from the reticulo-endothelial system and that the tumor cells exhibit capacity to differentiate into plasma cells, myelocytes, lymphocytes and even erythroblasts. According to this view, the tumor represents a form of totipotent myeloma capable of forming any one of the specific types of myeloma. This interpretation seems to have been rather widely accepted in Europe. The writer cannot accept this view and believes that the eminent French investigators have failed to distinguish between a specific type of endothelial tumor and other rarer round cell myelomas among which may probably be some that arise from the hemopoietic cell system or indifferent reticulum cells. The writer believes that the typical endothelioma of bone arises from capillary endothelium and never exhibits any other properties than those belonging to vascular endothelium. The pseudo-rosettes, the characteristic perithelial structures, and the cords of polyhedral cells lining elongated spaces are the outstanding structural features of this tumor and they never appear in any tumor derived from hemopoietic cells or reticulum cells. Plasma cells, granular leucocytes and lymphocytes are notably absent from tumors presenting these features, and when they are present the tumor should be excluded from the group of endothelioma. Moreover, in many cases of endothelioma there are associated with the above structural features, dilated blood channels of various sizes composed of the typical tumor cells, disclosing the angioblastic properties of the cells and connecting the tumor with other angiomas or angiosarcomas. Similar features are observed in capillary angiosarcomas of other organs, notably the skin."

The histology of the present case is consistent with Ewing's interpretation.

DISCUSSION

There are various features worth emphasizing in this case: (1) origin in the femur, the commonest site of Ewing's tumor, as reported by Geschickter and Copeland⁷ (incidence 28 per cent); (2) the short clinical duration of only 2 months; (3) the history of trauma at the onset which obviously brought to notice an already existing disease of the bone; (4) the solitary metastatic lesion despite large size of the primary mass; (5) the presence of

logic fractures, it would not have been possible to state whether it arose in bone or in adjacent soft tissues. Postmortem radiographs (Fig. 1) were reported as showing general decalcification of bone with worm-eaten structure, double pathologic fractures, very little evidence of reaction or repair and a large soft tissue extension of tumor. The appearance was regarded as consistent either with metastatic disease in bone or a rapidly growing primary osteolytic bone tumor.

The brain, abdominal viscera, thoracic viscera other than the right lung, and the soft tissues generally, showed no evidence of tumor. The adrenals were carefully investigated, with negative findings. The only tumor apart from the main mass consisted of a small nodule 1 cm. in diameter on the external surface of the base of the right lung. Aside from bilateral terminal pneumonic consolidation, no other lesions were demonstrated.

Histology of the Bone Tumor

The most prominent histological feature was the presence of a large number of pseudorosettes (Fig. 2). Between the pseudorosettes was seen a diffuse growth of uniform polyhedral cells. In places an occasional capillary blood vessel was surrounded by similar cells—the characteristic perithelial arrangement. One peculiar feature worth emphasizing was the lack of intercellular connecting fibers, the cells being quite separate from one another. The individual cells were uniform in size and shape. They appeared polyhedral. The nuclei were not hyperchromatic. There were no mitotic figures to be seen. The nuclear material took a homogeneous stain and the cytoplasm was very scanty. The histology was consistent with the diagnosis of endothelial myeloma of bone. Sections were stained for reticulum fibers by Foot's method and it was found that the cells of the tumor had no specific relation with the reticulum fibers. A few discrete reticulum fibers were seen.

Histogenesis

The histogenesis of Ewing's tumor is still under active debate. Oberling⁴ was the first to bring forward evidence that the growth is in reality a reticulosarcoma. According to him there are several histological types. The first has the structure of an undif-

DESCRIPTION OF PLATE

PLATE 96

FIG. 1. Roentgenogram, made after autopsy, showing a double fracture of the femur produced by a Ewing's tumor.

FIG. 2. Several closely approximated pseudorosettes in Ewing's tumor of bone. $\times 350$.

a fracture at two places, an unusual feature in Ewing's tumor; (6) an histological picture very unusual in respect to the large number of pseudorosettes in a single field.

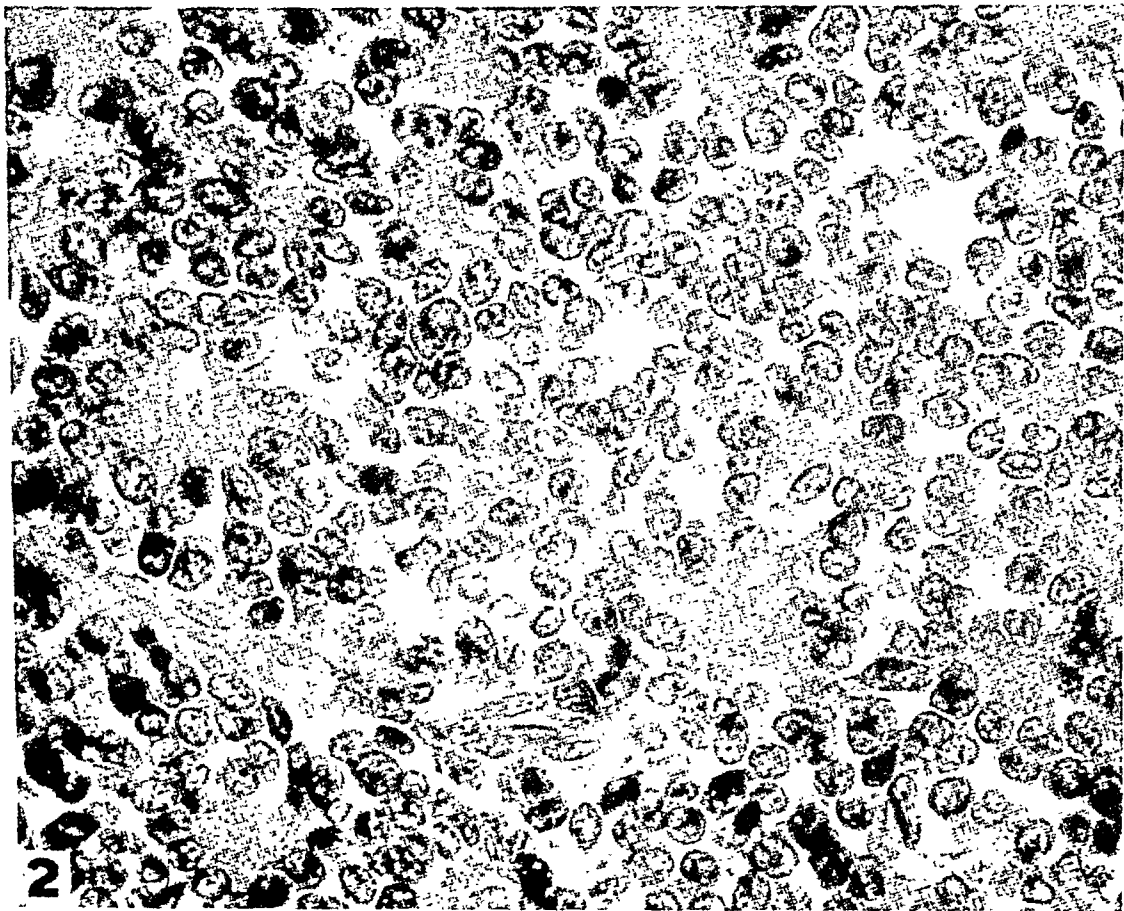
SUMMARY

A case of Ewing's tumor of bone is recorded in which complete autopsy revealed a single small pulmonary metastasis and nothing whatever which could be regarded as a primary neuroblastoma. Doubt, recently expressed, concerning the existence of the entity "Ewing's tumor of bone" is considered untenable.

NOTE: Grateful acknowledgment is made to S. R. Joglekar and P. V. Gharpure for the use of their material.

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period of injection was less than 3 weeks, while "in rats weighing about 190 to 250 grams, the potency of an extract can be evaluated in as short a time as two weeks." On the other hand, Silberberg,⁵ studying the endochondral ossification of the tibias of normal young guinea pigs (130 to 220 gm.), reported that after only 4 injections of acid extract of bovine anterior pituitary,* stimulation of cartilage and bone growth could be noted. Injections over a longer period of time caused a premature calcification and hence closure of the epiphyseal line. More recently, Freud, Levie and Kroon¹ have generalized their findings on the vertebrae, tibias and ribs and have stated that hypophysectomy in rats results in epiphyseal closure but that growth hormone injections, when instituted immediately following the operation, prevent this closure.

With the apparent contradictions in the reports on the response of the skeletons of rats and guinea pigs and because age is known to influence skeletal response in other disorders (*i.e.*, rickets), it was believed that this problem warranted further investigation.

MATERIAL AND METHOD OF PREPARATION

Forty female rats have been studied. These were divided into three age groups: approximately 54, 88 and 150 days of age at autopsy.

Each of these groups was subdivided into unoperated controls, unoperated injected animals, hypophysectomized controls, and hypophysectomized injected rats (Table I, groups A, B, C and D respectively). Females were chosen in order to eliminate sex factors and as far as possible litter mates were used in each age group. Following weaning, the animals were placed on a diet (Evans, No. XIV) consisting of 68 per cent ground whole wheat, 10 per cent fish meal, 10 per cent alfalfa leaf meal, 6 per cent casein, 5 per cent fish oil and 1 per cent NaCl. This diet has been tested on a series of 1100 females and 500 males and proved in this colony to result in growth comparable to that obtained on Diet 1, McCollum stock. At 28, 64, 125 and 140 days of age, respectively, half the animals in each group, chosen at random, were hypophysectomized by the parapharyngeal approach of

* It might be noted that the accepted method of extracting growth hormone is from an alkaline solution.

EFFECT OF THE PITUITARY GROWTH HORMONE ON THE EPIPHYSEAL DISK OF THE TIBIA OF THE RAT *

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INTRODUCTION

Recently Freud, Levie and Kroon¹ have suggested testing the potency of growth hormone preparations by means of tail growth and vertebral development in hypophysectomized rats. This method offers definite advantages both in simplicity and reliability over body weight increments which usually show considerable variation. However, before a biological method of assay such as this can be generally accepted, the influence of both intrinsic and extrinsic factors affecting the response must be established. Does the age of the animal have any influence? Is the response confined to the epiphyseal cartilage as claimed by Freud, Levie and Kroon? If so, do all the epiphyseal cartilages of the body react in the same manner? Furthermore, what is the effect of other endocrines, particularly those commonly found in alkaline extracts of the anterior pituitary, on the skeleton? Do any of these synergize or antagonize the action of growth hormone? This preliminary investigation deals with the first of these questions, the purpose being to determine the influence of *age* on the response of the skeleton to growth hormone injections in both unoperated and hypophysectomized rats.

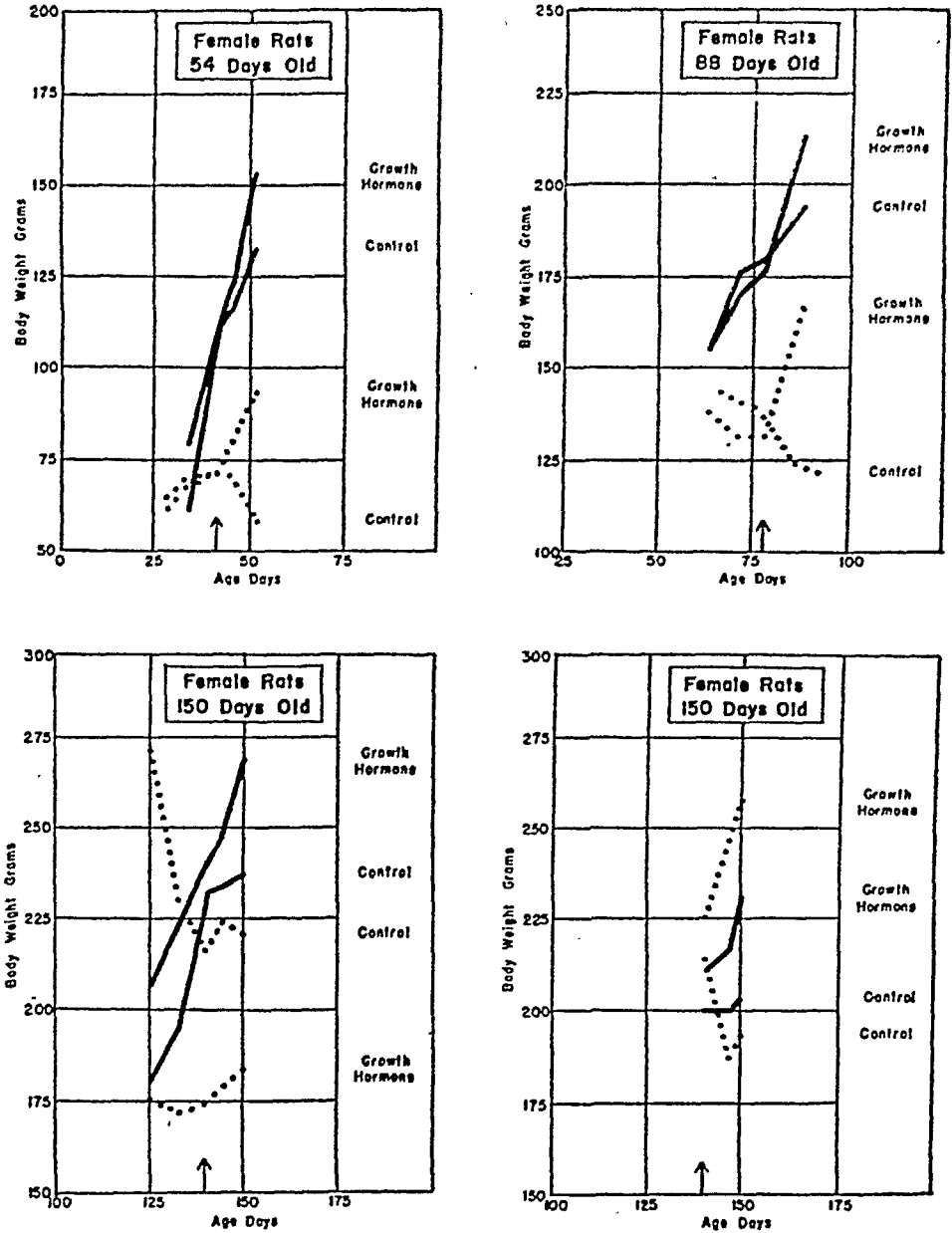
A specific relation of pituitary derangements to skeletal growth has been suspected clinically for some time. In 1921, Evans and Long² first demonstrated the effects of anterior pituitary extracts on body weight as a whole. Dott and Fraser³ in 1923 showed a definite skeletal relation experimentally in a study on dogs and cats. Later, Handelsman and Gordon,⁴ using the skull and mandible of the rat as "test bones," studied age differences in response to growth hormone injections and reported that animals under 90 days of age showed little or no stimulation of bone growth if the

* Read before the San Francisco Section of the International Association for Dental Research, San Francisco, California, February 2, 1939.

Summary presented at the Convention of the American Association for the Advancement of Science, Columbus, Ohio, December 29, 1939.

Received for publication December 21, 1940.

Smith.⁶ The weight changes were followed for 2 weeks and if incompleteness of the operation was suspected, the animals were discarded. Completeness of hypophysectomy was further verified at autopsy. Following this 2-week period, the animals to be treated were given 10 daily intraperitoneal injections of growth



Legend

- Unoperated Animals
- Hypophysectomized Animals (Curve starts on day of operation)
- Onset of Injection

TEXT-FIGURE 1. Average body weights plotted against the age in days. Curves for hypophysectomized animals start on the day of operation.

TABLE I
Distribution of Materials

Group	Age (days)	Post- operative days	Rat No.	Injection	
				No. of days	Total solid (mg.)
A. Unoperated controls	54		W 30		
	54		BH55		
	54		W 82		
B. Unoperated injected	54		W 29	10	14.4
	54		G 34	10	14.4
	54		W 53	10	14.4
C. Hypophysectomized controls	54	25	G 32		
	54	25	W 79		
D. Hypophysectomized injected	54	25	G 33	10	8.8
	54	25	W 51	10	8.8
	48	19	GH84	4	1.4
A. Unoperated controls	88		BH09		
	88		BH77		
			BH94		
B. Unoperated injected	88		BH08	10	68.7
	88		W 72	10	68.7
			BH92	10	68.7
C. Hypophysectomized controls	88	25	W 75		
	92	25	BH05		
	92	25	BH33		
	92	25	BH43		
D. Hypophysectomized injected	88	25	W 03	10	41.9
	88	25	W 73	10	41.9
			W 90	10	41.9
A. Unoperated controls	150		W 97		
	150		W 07		
	150		B 15		
	150		W 23		
B. Unoperated injected	150		B 53	10	84.2
	150		W 09	10	68.7
	150		W 24	10	68.7
	150		B 44	10	68.7
C. Hypophysectomized controls	150	25	W 88		
	150	11	B 27		
	150	11	B 14		
	150	11	B 42		
D. Hypophysectomized injected	150	25	W 58	10	54.7
	150	11	G 10	10	41.9
	150	11	B 16	10	41.9
	150	11	BH45	10	41.9

calibrated with a stage micrometer. The cortical bone (periosteal ossification) was measured in the proximal region of the diaphysis next to the fibula, the articular cartilage (hyaline cartilage proliferation) in the center of the articular surface, and the epiphyseal line (endochondral ossification) in the central portion.

In addition to the rats already mentioned, serial sections were made also of the proximal portion of the right tibias of an additional group of normal animals (Table II). In these, every 20th section was photographed and a definite area in the tibia determined for all subsequent comparisons. This area in the group 54 days old was in the central portion of the lateral articular surface just medial to the fibula; in the other two groups it was in the central portion of the tibia between the two articular surfaces.

RESULTS

In the young rat the epiphyseal disk may be divided into four definite zones, from the epiphysis toward the shaft:

1. Remnants of the embryonic hyaline cartilage with the cells irregularly arranged (Fig. 1, a).
2. Proliferating basophilic cells arranged in columns parallel with the long axis of the shaft (Fig. 1, b).
3. Vesicular cells, occupying large lacunae, also arranged in columns (Fig. 1, c).
4. Line of "erosion" where capillary loops from the vascular bed of the diaphysis meet the advancing rows of cartilage cells (Fig. 1, d).

Early in the life of a rat (between 24 and 54 days of age) an equilibrium is established in the tibia between the proliferation of cartilage and endochondral ossification at the epiphysis. This is shown graphically in Text-Figure 2. During this early period the original embryonic cartilage is rapidly replaced by bone and marrow until all that remains is the epiphyseal disk as found in the adult. From this period on there is a gradual reduction in the width of both the basophilic and vesicular zones (Text-Fig. 2 and Table II) but the proximal epiphyseal disk of the tibia persists until senility (Dawson⁹). A balance between deposition and resorption is also established in the cortical bone before 74 days of age and in the articular cartilage prior to 30 days of age

hormone. The animals 140 days of age at hypophysectomy were injected on the day of operation. The foregoing data are summarized in Table I.

The growth hormone was prepared by Uyei⁷ and had previously been standardized so that "acute" injections could be given, *i.e.*, double the amount of hormone necessary to give maximal growth. The total amount of hormone in milligrams of solid material injected during the 10-day period is also given in Table I.

The weight response was marked in the hypophysectomized animals of each age group (Text-Fig. 1). In the unoperated rats, this response varied considerably with the age, the difference between control and injected rats being greatest in the oldest group, least in the youngest. The curves in Text-Figure 1 represent the average body weights of the animals in each group as plotted at 5-day intervals during the experimental period. The increases in weight were not always constant so the figures for each animal have been included in Tables III to V ("Gain in body weight").

The right tibia of each animal was decalcified, embedded in nitrocellulose and sectioned serially at 8 to 9 μ . Three staining procedures were employed: Böhmer's hematoxylin and eosin, Mallory's azan, and a new stain developed by Koneff⁸ for differentiating hyaline cartilage, calcified cartilage, preosseous substance and mature bone. Measurements made with an eyepiece micrometer are given in arbitrary units. Later the ocular was

TABLE II
Normal Rats: Summary of Measurements

Total number of rats	Age	Average body weight	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
	(days)	(gm.)			
1	24	47	13	23	127
1	28	61	12	17	102
3	54	135	15	13	38
1	74	188	28	14	63
3	88	196	20	15	31
2	90	240	22	15	28
1	100	186	23	15	48
1	123	194	26	13	38
4	150	212	25	11	40
1	170	213	26	15	45
1	305	250	22	10	27
1	384	250	25	14	17
1	406	268	20	14	23

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ .

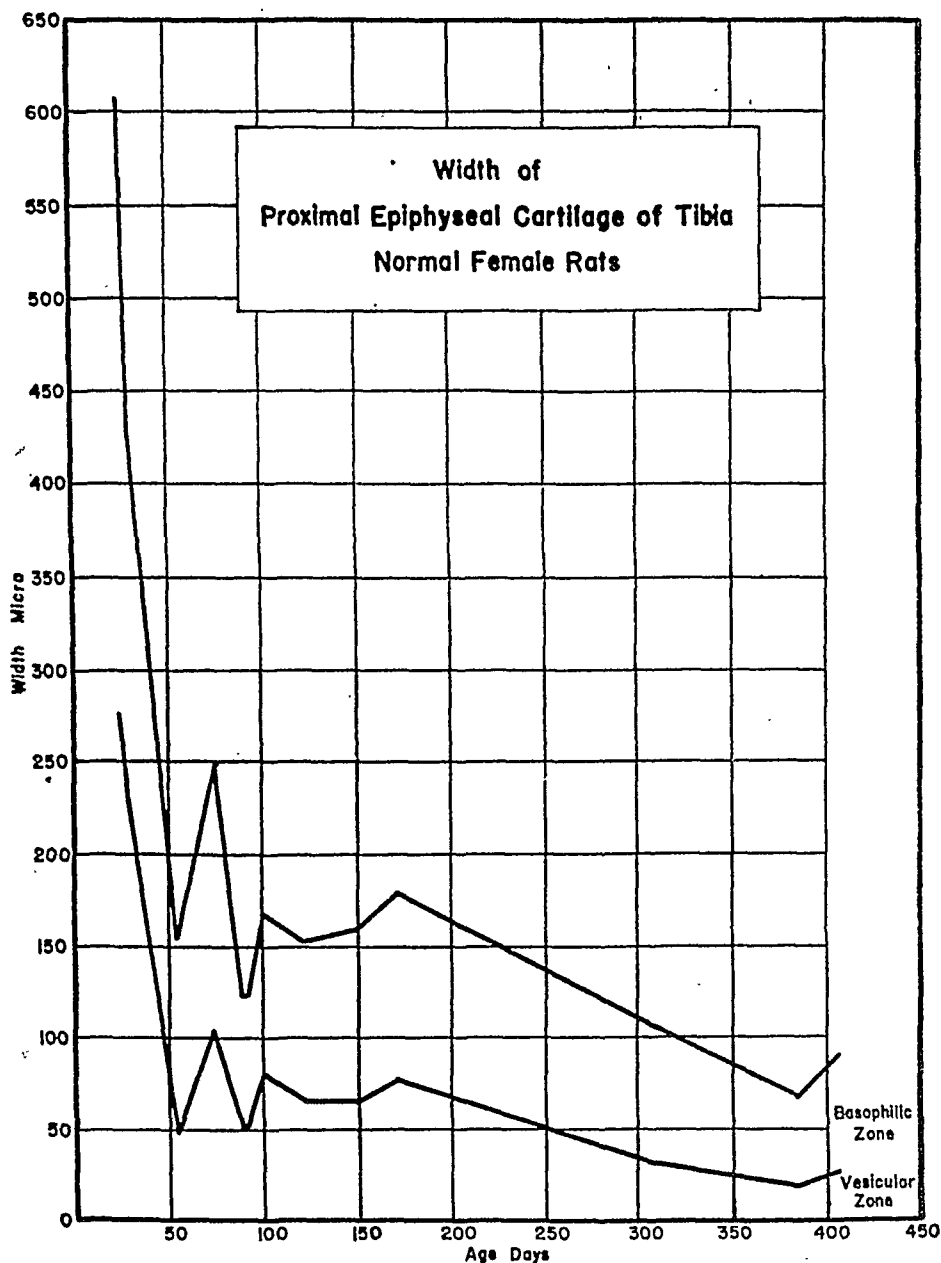
† One division equals 4.1 μ .

tibia is established. The middle group, 88 days of age, represents active but mature bone growth. The oldest group, 150 days of age, is representative of the suspended growth of the adult rat.

Histologically, the equilibrium established in endochondral ossification is less stable and hence more sensitive than that in periosteal and endosteal ossification. The embryonic hyaline cartilage is present as a continuous zone up to 50 days. At 88 days of age it is absent in places and by 150 days only scattered traces remain. Mitotic figures are frequent in the basophilic cells of the young rat but in animals 150 days old they were not observed. With advancing age there is a reduction in the length of the columns, and an increasing irregularity in their arrangement as well as an increase in the irregularity of the width of the disk as a whole (Fig. 2). These changes are accompanied by a decrease in the size and number of the individual cells and a relative increase in the amount of matrix (Figs. 6, 10 and 14). With the decrease in width of the vesicular zone (Text-Fig. 2) there is also a reduction in the size of the cells; the nuclei become somewhat more basophilic, and the columnar arrangement is less regular. Activity along the zone of erosion decreases with age and in old animals (150 days and over) bone is deposited along this region, thus effectively sealing the epiphyseal cartilage from the diaphysis. In young growing rats, however, longitudinal trabeculae of cartilage protrude into the medullary cavity of the shaft as growth proceeds and the surfaces become covered with a thin layer of preosseous substance. More distal in the diaphysis, the cartilage is gradually replaced by preosseous substance and finally by fully formed bone.

To summarize the histological changes in the epiphyseal disk of the normal rat tibia, with increasing age one observes:

1. Increasing irregularity in total width of disk.
2. Disappearance of embryonic hyaline cartilage.
3. Increasing irregularity in arrangement of cells in basophilic and vesicular zones.
4. Reduction in size and number of cells in both of these zones.
5. Lack of activity and subsequent deposition of bone along the line of erosion.



TEXT-FIGURE 2. Variation in the width of the epiphyseal cartilage with age.

(Table II). The establishment of these equilibria does not mean that skeletal growth ceases but that ossification keeps pace with cartilage formation and that remodeling accompanies periosteal ossification.

From Text-Figure 2 the age groups chosen for this experiment can readily be placed. The youngest group, 54 days of age, comes at the end of the period during which the epiphyseal plate of the

However, the epiphyseal cartilage was decreased in width in one animal (Table III, group C, W79).

The histological changes in the epiphyseal line shown in Figure 12 were both constant and marked in all the hypophysectomized rats of this group. The cells of the zone of proliferation were small, their nuclei pyknotic and they were reduced in number. The columns were regular in arrangement but much narrower than in the unoperated rats and thus there was a relative increase in the amount of matrix between them. The cells of the vesicular zone appeared shrunken, the lacunae small and there was a consequent reduction in the width of the zone. The erosion zone was inactive. In the diaphysis, the disappearance of the trabeculae was very striking. Their disappearance cannot be explained as a cessation of growth; there was an actual resorption of bone, but with no increase in the number of osteoclasts. The remaining trabeculae were large and well oriented. The marrow showed a tremendous increase in fat content at the expense of the myeloid elements.

Effect of Growth Hormone on Hypophysectomized Rats

The response of the hypophysectomized animal to growth hormone injections was very much greater than that of the unoperated rat. The cortical bone had a tendency toward increased thickness (Table III, compare groups C and D, column 1). The average width of the epiphyseal cartilage was even greater than in the unoperated injected rats (Table III, groups A and D, column 3); compared with the hypophysectomized controls (group C), the average increase in width was 53 units.

The histological differences between these animals and their operated controls were still more marked than were the differences in measurements (Fig. 13). The cells of the hyaline cartilage covering the epiphysis and their lacunae were larger, the nuclei were karyolytic, the cytoplasm vesicular and the cells as a whole less basophilic than in the hypophysectomized controls. The number of cells in the zone of proliferation of the epiphyseal cartilage increased tremendously with subsequent increase in length of the columns. The matrix became less homogeneous in character, taking the hematoxylin in streaks of blue. The cells of the vesicular zone and their containing envelopes of matrix were larger; the

RAT TIBIA AT 54 DAYS

Effect of Growth Hormone on Unoperated Rats

The measurements of the various regions of the tibias of the unoperated rats, aged 54 days, are presented in Table III. In comparing groups A and B, the cortical bone and articular cartilage showed no changes in thickness following 10 injections of growth hormone. A slight increase in width of the epiphyseal cartilage was not great enough to be significant.

TABLE III
Rats 54 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
A. Unoperated controls	W30	(gm.) 30	17	12	40
	BH55	8	15	15	32
	W82	28	15	12	43
	Average	22	16	13	38
B. Unoperated injected	W29	38	12	15	45
	GH34	34	17	14	44
	W53	34	12	13	36
	Average	32	14	14	42
C. Hypophysectomized controls	G32	— 2	13	15	27
	W79	— 16	11	..	14
	Average	— 9	12	15	20
D. Hypophysectomized injected	G33	20	15	13	90
	W51	24	14	14	74
	GH84	5	14	16	55
	Average	16	14	14	73

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ .

† One division equals 4.1 μ .

The survey pictures showed a gross similarity between the control and injected rats. However, at higher magnification (Figs. 10 and 11) a definite increase in activity along the zone of erosion, following the injections, can be discerned. There is also a reduction in the size of the trabeculae with an increase in their number, and a slight decrease in the number of fat cells of the marrow.

Effect of Hypophysectomy

After a postoperative period of 25 days there was no significant change in width of the cortical bone or of the articular cartilage.

were frequently lacking. The transition between the zone of proliferation and that of the vesicular cells was less abrupt, the karyolytic changes in the nuclei proceeding more gradually, especially in the peripheral areas of the disk. The lacunae of the vesicular cells were larger on the whole and the zone wider than in the controls, but the average number of cells was the same (See Figs. 2 and 9).

TABLE IV
Rats 88 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical bone width*	Articular cartilage width*	Epiphyseal cartilage width†
A. Unoperated controls	BH09	(gm.) 27	16	11	33
	BH77	6	19	..	27
	BH94	20	25	19	34
	Average	18	20	15	31
B. Unoperated injected	BH08	38	20	13	36
	W 72	43	20	13	41
	BH92	32	24	13	36
	Average	38	21	13	38
C. Hypophysectomized	W 75	— 2	17	9	21
	BH05	— 7	17	11	14
	BH33	— 12	23	13	16
	BH43	— 6	15	13	19
	Average	— 7	18	12	18
D. Hypophysectomized injected	W 03	30	20	10	64
	W 73	42	18	7	74
	W 90	34	18	12	78
	Average	35	19	10	72

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ .

† One division equals 4.1 μ .

Effect of Hypophysectomy

There was a slight decrease in the average thickness of both the cortical bone and the articular cartilage in the animals of this group following the postoperative period of 25 days (Table IV, columns 1 and 2, compare groups A and C). The decrease in average width of the epiphyseal line (column 3) was 13 units, which was somewhat greater than the decrease in the younger animals. Figure 4 is a survey picture typical of the animals of this group. It shows the gross changes in the epiphyseal disk after hypophysectomy.

The histological changes in the disk are shown in Figure 8.

nuclei of the cells were either karyolytic or entirely lacking. The erosion zone was extremely active and packed with cells, in marked contrast to the same zone in the hypophysectomized controls. The diaphysis was filled with numerous small trabeculae that were covered with osteoblasts, and osteogenesis as well as chondrogenesis was extremely active. The marrow recovered from the effects of hypophysectomy with a reduction in the number of fat cells and an increase in the myeloid components.

The rapidity and sensitivity of the response of the hypophysectomized rat to growth hormone injections was shown in the case of one animal (Table III, group D, GH84) that died because of alizarin red injections at 48 days of age after only 4 injections of the hormone. The histological changes in the tibia of this rat were comparable in all respects to the changes in the bones of animals injected over the 10-day period. However, the gain in body weight was only 5 gm. as compared with 20 and 24 gm. increases for the other two animals in the same group.

RAT TIBIA AT 88 DAYS

Effect of Growth Hormone on Unoperated Rats

The measurements of the various regions of the tibia are presented in Table IV. The cortical bone and articular cartilage showed no pronounced changes in width (compare groups A and B, columns 1 and 2). The average increase in width of the epiphyseal disk (column 3) was 7 units, again showing a very slight difference. There is no correlation in either this group or in that of animals 54 days old between the total body weight and the width of the epiphyseal line. The gross changes are shown in the survey pictures (Figs. 2 and 3).

The histological differences between the injected and the control animals of this group were more marked than in the preceding group (compare Figs. 6 and 7). In the unoperated animals of the group 54 days old, changes following injections were confined to the zone of erosion but in these animals there were also noticeable differences in the epiphyseal cartilage. The zone of proliferation was wider in the injected animals; the cells increased correspondingly both in number and size and were less basophilic. Toward the epiphyseal end of the columns the cells became so closely packed that the transverse matrix septa between them

TABLE V
Rats 150 Days Old: Summary of Measurements

Group	Rat No.	Gain in body weight, 10 days	Tibia, proximal portion		
			Cortical width* bone	Articular cartilage width*	Epiphyseal cartilage width†
A. Unoperated controls	W97	(gm.) 6	25	11	40
	W07	0	24	..	18
	B15	6	24	..	20
	W23	2	25	10	18
B. Unoperated injected	B53	42	24	14	55
	W09	23	21	12	25
	W24	20	20	..	25
	B44	18	22	..	28
C. Hypophysectomized controls	W88	4	22	14	27
	B14	-30	23	..	12
	B27	-16	18	13	13
	B42	-16	22	..	15
D. Hypophysectomized injected	W58	10	20	8	56
	G10	23	..	14	50
	B16	-14	22	..	28
	BH45	16	25	..	27

* Measured with a calibrated ocular. One division is equivalent to 16.1 μ .
 † One division equals 4.1 μ .

The histologic changes were the same in both groups. There were no essential differences in the cortical bone and articular cartilage between control and treated animals. The changes in the epiphyseal cartilage are shown in Figure 15. It was hypertrophied in all zones and much more regular in width. The cells in the zone of proliferation were larger, their nuclear detail was clearer and frequently several cells occupied a single lacuna—evidence of the rapidity of proliferation. The lacunae of the zone of vesicular cells were also enlarged, the matrix between them less abundant and less basophilic than in the controls. The nuclei of the cells were large and possessed two distinct nucleoli; their cytoplasm was abundant and "lacy" in character. Activity along the zone of erosion was confined to a narrow band which was more or less clearly set off from the rest of the medullary cavity. This line of activity was evident in these animals because of the relative inactivity of the bone prior to the period of injections; on the other hand, in younger animals such a band could not be clearly discerned because of the activity previous to the injections. As in

The picture of regression in the disk and bone atrophy is the same as previously described for the younger group, but it is more marked in degree.

Effect of Growth Hormone on Hypophysectomized Rats

There was a decrease in width of the articular cartilage not found in the rats 54 days old (Table IV, group D, column 2), but there was no change in the width of the cortical bone (column 1). The average increase in thickness of the epiphyseal cartilage over the operated controls was 54 units (column 3, compare groups C and D), an even greater increase than in the younger animals. A comparison of the survey pictures, Figs. 4 and 5, will show the magnitude of the response of these rats to the injections.

The histological changes in the epiphyseal cartilage, diaphysis and marrow shown in Figure 9 were identical in type with those already described for the hypophysectomized animals, 54 days old, injected with growth hormone, but the differences as compared with the control, Figure 8, were also more pronounced.

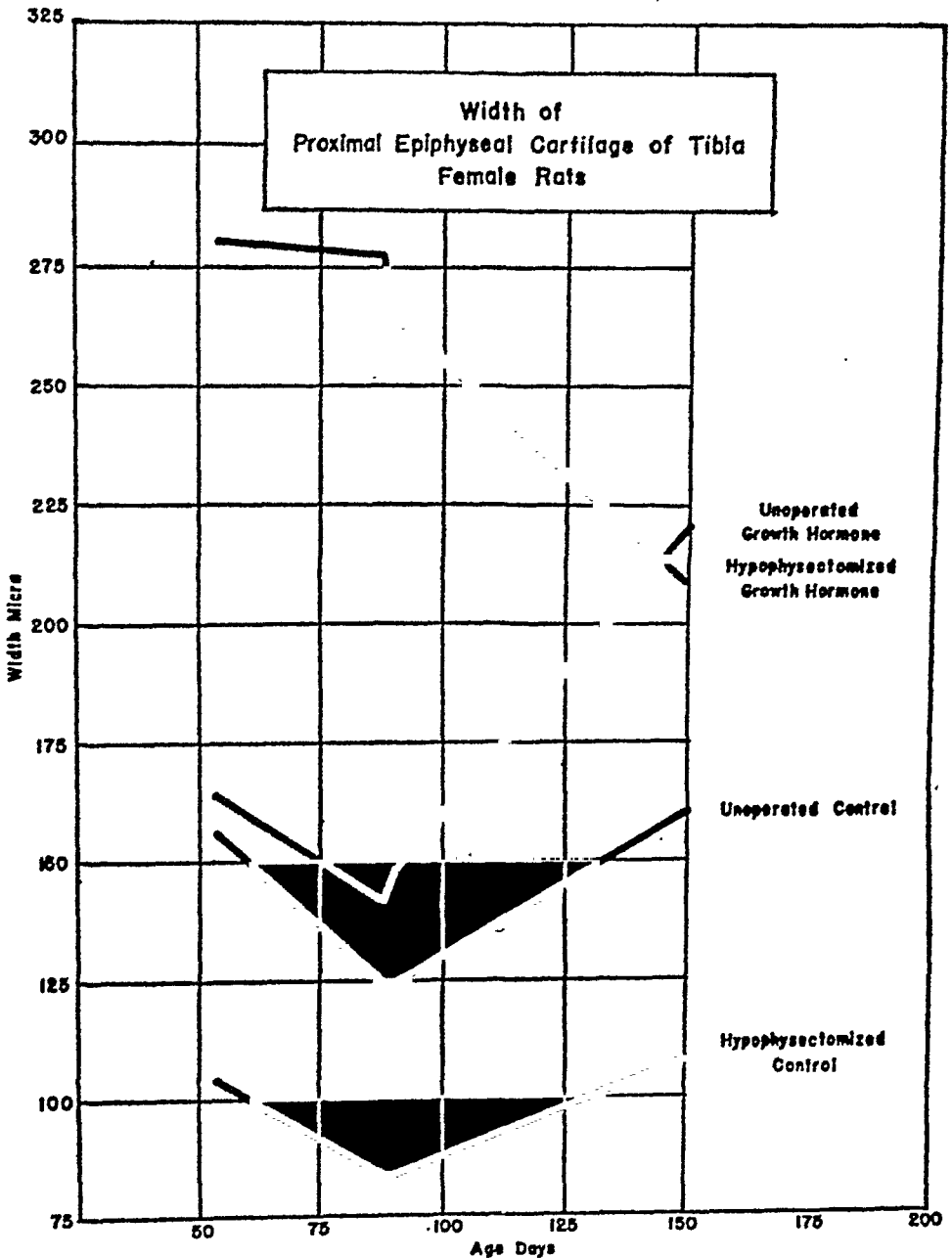
RAT TIBIA AT 150 DAYS

Effect of Growth Hormone on Unoperated Rats

The first group of animals 150 days old which was studied showed changes in the width and staining reaction of the epiphyseal disk that did not correspond with those found in the two groups previously described. Since it was felt that the difference was due to faulty technic in fixation and decalcification, a second series was prepared. The first animals in groups A, B, C and D, Table V (W97, B53, W88 and W58) belong to this series and were chosen as representative from a total of 15 rats. The following discussion will apply primarily to them. The remainder of the animals in Table V belong to the first group and must be considered separately as far as measurements are concerned.

The cortical bone (Table V, column 1) did not show any pronounced changes in thickness; there was a slight but inconclusive increase in the average width of the articular cartilage (column 2). The epiphyseal cartilage (column 3) was markedly increased in thickness in the first group, and increased to a less pronounced degree in the second (last three animals of groups A and B).

line such as this was not observed in the younger animals. It probably corresponds to the "line of arrested growth" common in many pathological processes as described by Harris.¹⁰ The response of the epiphyseal cartilage and the increase in activity along the zone of erosion are shown in Figure 16. A final point of interest in this group was that one animal (Table V, group D, Br6) continued to lose weight during the period of injection and yet showed pronounced stimulation of endochondral ossification.



TEXT-FIGURE 3. Average width of the proximal epiphyseal cartilage of the tibia plotted against the age (54, 88, 90 and 150 days) at autopsy.

the groups of animals already described, there was a definite stimulation of the myeloid elements of the marrow at the expense of the fat cells.

Effect of Hypophysectomy

The first animals in groups C and D, Table V (W88 and W58) had been hypophysectomized for 25 days, the remaining 6 for a period of only 10 days; hence the difference in the loss of weight of the controls. There was a tendency toward a decrease in the width of the cortical bone and an increase of the articular cartilage. The measurements of the epiphyseal cartilage tended to lose their significance since the disk was very irregular in width.

The epiphyseal cartilage was distinctly outlined on *both* sides by a layer of bone. The histological changes in this region are shown in Figure 16. The matrix in the zone of proliferation was relatively abundant because of the reduction in both the size and the number of cells. The cells themselves were extremely basophilic, the nuclei so much so that their detail could not be made out. The vesicular zone was very narrow and here even the matrix was basophilic. The cells of this zone were almost as small and basophilic as the cells of the preceding zone and in places there appeared to be transitions of these cells into osteocytes. The zone of erosion was lacking, and instead bone had been deposited in this region. In the diaphysis, the trabeculae were reduced in number, although the atrophy was not so marked as in the animals 88 days old.

Effect of Growth Hormone on Hypophysectomized Rats

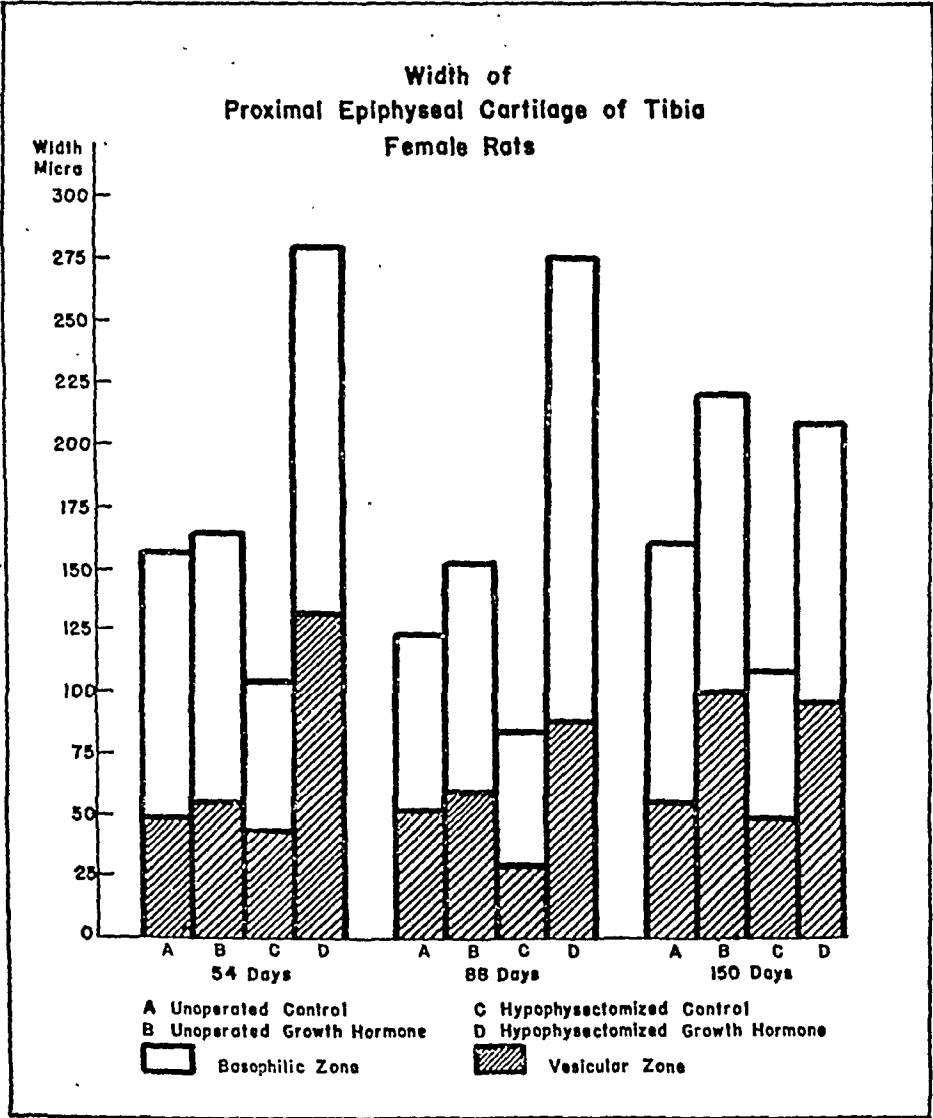
The measurements of the cortical bone and articular cartilage did not show any definite trends (Table V, group D, columns 1 and 2). The increase in width of the epiphyseal cartilage (column 3) was marked and could readily be seen in the survey picture, but it was nowhere near so great as in the rats 54 and 88 days old.

Histologically, the changes were essentially the same as in the injected, unoperated animals of the same age group. Previous to the injections, bone had been deposited along the diaphyseal side of the epiphyseal cartilage. This layer clearly delimited the activity, following injections, from the rest of the diaphysis, although it did not prevent the cartilage from responding. A clear

physeal cartilage was proportional in all three age groups, indicating, together with the histological changes, an active process rather than a passive cessation of growth. If the latter were the case one would not expect to find such distinct changes in skeletally mature animals. The response of hypophysectomized animals to injections was much more pronounced than in the unoperated rats. This may indicate that some fraction normally present in the pituitary antagonizes the action of growth hormone. Finally, the response of the epiphyseal cartilage in the operated rats, in contrast to that in the unoperated animals, was less marked at 150 days of age than at 50, which may be interpreted as a reduction in sensitivity with increasing age.

These observations differ in several respects from those of Freud, Levie and Kroon,¹ who reported that growth hormone primarily affects the proliferating cartilage. The fraction used in this laboratory also caused marked stimulation of endochondral ossification and coincident connective tissue metaplasia. In addition, definite stimulation of the myeloid components of the marrow followed injections. Freud, Levie and Kroon also reported that the effects of hypophysectomy were localized in the epiphyseal cartilage and that epiphyseal closure followed the operation. However, Figure 4 shows, in addition to changes in the disk, definite atrophy of the trabeculae and marked increase in the fat content of the marrow.* The deposition of bone along the epiphyseal cartilage described by Freud, Levie and Kroon depends to some extent on the age of the animal, but even in rats 150 days old after a postoperative period of 2 weeks this did not prevent the response of the disk to injections. Following a postoperative period of 3 to 4 weeks there was still no definite evidence of "irreversible" epiphyseal closure. It may be concluded that the action of the anterior pituitary fraction used in this laboratory and also the effect of hypophysectomy are not confined to the epiphyseal disk but influence the whole process of endochondral ossification and coincident connective tissue metaplasia in the tibia. The effect of this fraction on normal periosteal ossification is less marked, which may explain why

* It has been reported by Gaebler¹¹ and Evans, Luck, Pencharz and Stoner¹² that hypophysectomy raises the respiratory quotient due to decreased utilization of fats. The reverse takes place following growth hormone injections. This may be the biochemical explanation for the histological findings in the marrow.



TEXT-FIGURE 4. Average width of the proximal epiphyseal cartilage of the tibia at the ages indicated.

DISCUSSION

Text-Figures 3 and 4 summarize graphically the data on the widths of the epiphyseal cartilage (Tables III to V).

The greatest difference in width between normal control and injected animals came at 150 days of age. This may indicate an increase in the sensitivity of the response with advancing age, an observation to be expected since skeletal growth is already so active in young rats that it would be difficult to stimulate it further. Following hypophysectomy the reduction in width of the epi-

hypophysectomized animals (15 days postoperative + 10 daily injections = 25 days) are followed by (a) definite disruption of the equilibrium with increased cartilage formation and a return to the "youthful" type of epiphyseal line, most pronounced in young animals (54 to 88 days of age); (b) increased activity in the diaphysis with the formation of trabeculae and the deposition of bone along them, and (c) stimulation of the myeloid elements of the marrow with a reduction in the number of fat cells.

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DESCRIPTION OF PLATES

PLATE 97

FIG. 1. Epiphyseal disk of a normal adult rat. (a) Remnant of embryonic hyaline cartilage; (b) zone of basophilic cells; (c) zone of vesicular cells; (d) zone of erosion.

Handelsman and Gordon⁴ were unable to get a more sensitive reaction in the skull and mandible of the rat.

Silberberg's⁵ observation that epiphyseal closure soon follows the onset of injections may be explained on the basis of the fraction used. An acid extract of the anterior pituitary would probably be rich in gonadotrophic fractions which may cause calcification of the epiphyseal line.

The data previously presented (Tables III to V) tend to confirm the observations of Freud, Levie and Kroon¹ that the response of the epiphyseal cartilage of the tibia to growth hormone is much more constant than the gain in body weight. An extreme illustration of this is one of the hypophysectomized rats, aged 150 days, which continued to lose weight during growth hormone treatment (Table V, B16) but which showed changes in the tibia comparable with the other animals in the same group. Furthermore, the response of the tibia was much more rapid and sensitive than the increase in body weight, as previously pointed out for an hypophysectomized rat 54 days old (Table III, GH84) that died after only 4 injections.

CONCLUSIONS

1. Early in the life of a normal female rat (between 25 and 50 days of age) there is an equilibrium established between the formation of cartilage and bone in endochondral ossification, an equilibrium which, in the proximal end of the tibia, is maintained until very late in the life of the animal.

2. Injections of growth hormone over a short period (10 days) in normal animals are followed by definite stimulation of endochondral ossification with little disturbance of this equilibrium, except in rats 150 days old in which there is hypertrophy of the epiphyseal cartilage. Injections are followed also by a decrease in the fat content of the marrow.

3. Hypophysectomy is followed after 25 days not only by (a) disturbance of this equilibrium with a reduction in the width of the epiphyseal cartilage; but also by (b) resorption of the diaphyseal trabeculae; (c) an increase in the fat content of the marrow; and (d) in animals 150 days old, a deposition of bone along the diaphyseal side of the epiphyseal cartilage.

4. Injections of growth hormone over a similar period in

PLATE 98

FIGS. 2 to 5. Survey photomicrographs of the proximal epiphysis of the tibias of female rats, 88 days of age at autopsy.

FIG. 2. Control animal (BH09).

FIG. 3. Experimental animal (W72) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 4. Hypophysectomized control animal (W75), 25 days postoperative.

FIG. 5. Hypophysectomized animal (W73), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.

FIGS. 6 to 9. Changes in the central area of the epiphyseal disk in female rats 88 days of age at autopsy.

FIG. 6. Control animal (BH09).

FIG. 7. Experimental animal (W72) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 8. Hypophysectomized control (W75), 25 days postoperative.

FIG. 9. Hypophysectomized animal (W73), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.



Ray, Evans and Becks

Effect of Pituitary Growth Hormone

PLATE 99

FIGS. 10 to 13. Changes in the central area of the epiphyseal disk in female rats, 54 days of age at autopsy.

FIG. 10. Control animal (W82).

FIG. 11. Experimental animal (W53) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 12. Hypophysectomized control (G32), 25 days postoperative.

FIG. 13. Hypophysectomized animal (G33), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.

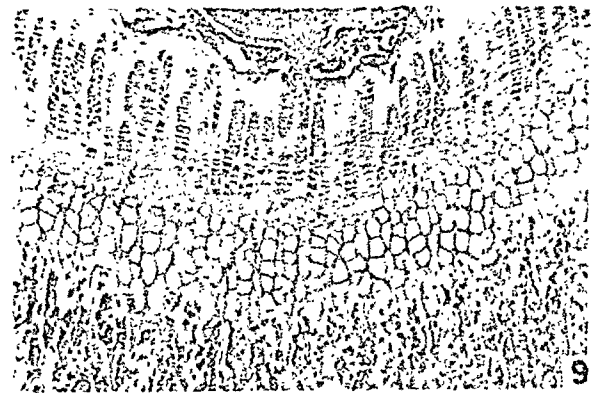
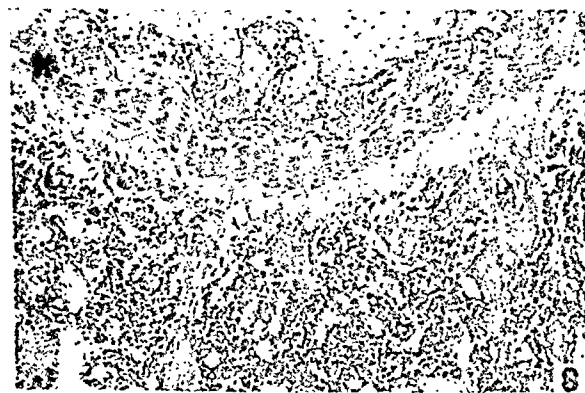
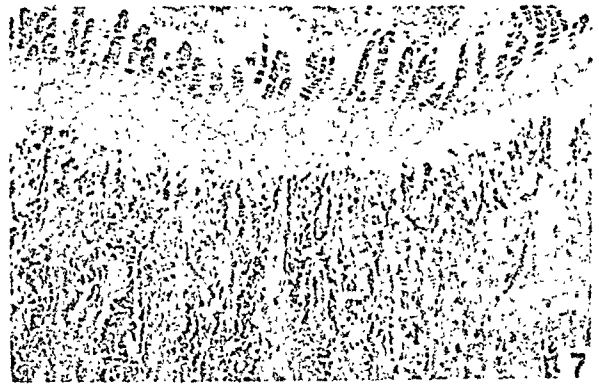
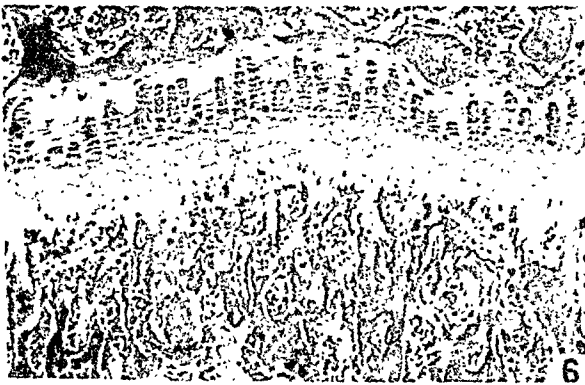
FIGS. 14 to 17. Changes in the central area of the epiphyseal disk in female rats, 150 days of age at autopsy.

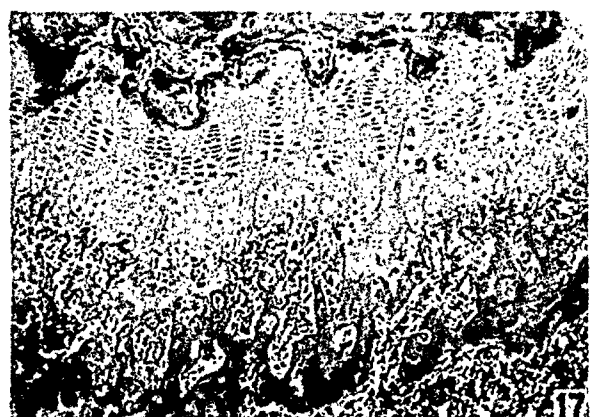
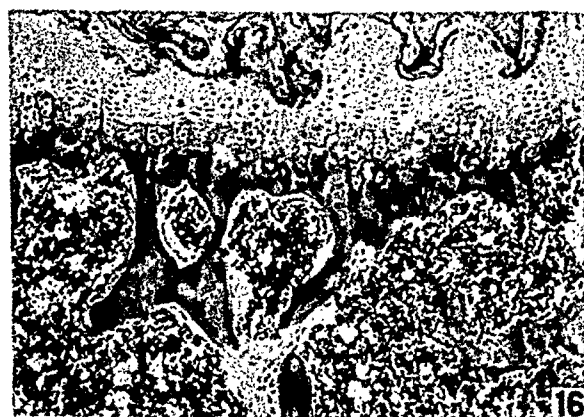
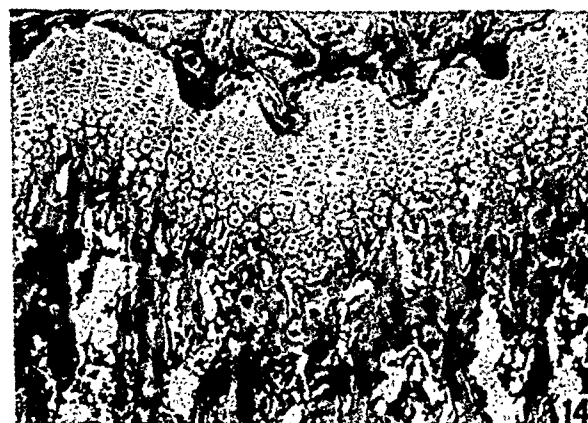
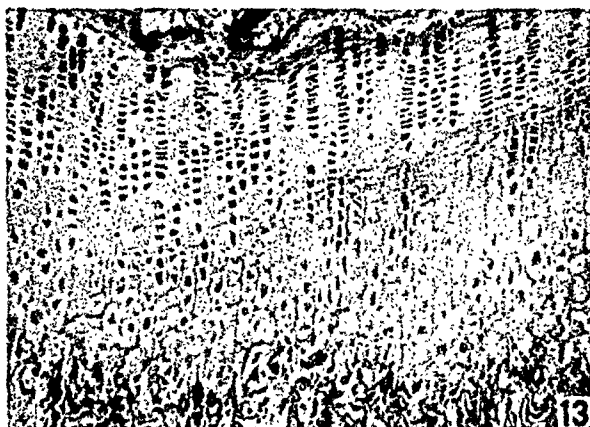
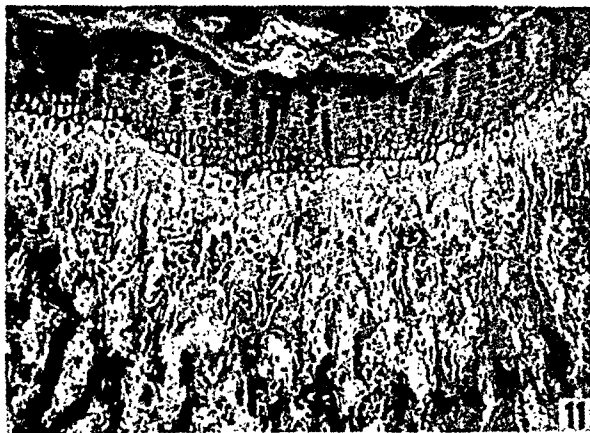
FIG. 14. Control animal (W97).

FIG. 15. Experimental animal (B53) receiving ten daily injections of growth hormone prior to autopsy.

FIG. 16. Hypophysectomized control (W88), 25 days postoperative.

FIG. 17. Hypophysectomized animal (W58), 25 days postoperative, given ten daily injections of growth hormone prior to autopsy.





deposited. The epithelial cells in the pulp were surrounded by dentin, which in turn was surrounded by odontoblasts. Excessive local formation of atypical dentin increased with the recovery period. Morphological recovery was complete in 19 days.

Wolbach and Howe² used a diet that was deficient in vitamins A, C, D and E, but indicated that the lack of vitamins C, D and E was insignificant in respect to complicating the vitamin A-deficiency picture in the rat. They found that the addition of vitamin A alone was sufficient to bring about histological recovery. In a later report Wolbach³ gave the following summary on the teeth in vitamin A deficiency: "The continuously growing incisor teeth of rodents—rats and guinea-pigs—are profoundly affected owing first to atrophy and metaplasia of the enamel forming organ and subsequently to atrophy and cessation of or irregular functioning of odontoblasts. Enamel formation is suppressed, and striking deformities of the dentin result."

Boyle⁴ described, in the tooth germ of a human infant with vitamin A deficiency, changes in the enamel organ which were similar to those found by Wolbach and Howe² in the rat incisor. Mellanby and King⁵ found hyperplasia of the gingivae and periodontal disease in dogs, rabbits and rats placed on a diet deficient in vitamin A. King⁶ confirmed previous findings in dogs, and in addition reported retarded eruption and malposition of the teeth; malformation of the roots associated with hypoplastic changes in Hertwig's epithelial sheath; ill-defined laminae durae; changes in alveolar bone and a tendency to apical hypercementosis. King⁷ also studied the effects of vitamin A deficiency in the rat and emphasized the disturbance in the calcification of dentin in spite of the fact that the animals were given ample amounts of vitamin D, calcium and phosphorus. Smith and Lantz⁸ reported a loss of normal pigment and a dull white, opaque appearance of the incisors of rats placed on a vitamin A-deficiency ration. The teeth were short and blunt. Eruption was markedly retarded. Fridericia and Gudjonsson⁹ also reported progressive retardation in the eruption of the incisors of rats in vitamin A deficiency. Orten, Burn and Smith¹⁰ studied the effects of prolonged incomplete vitamin A deficiency in the incisor of the white rat. They reported tumor growths (odontomata) which arose from the pulp and proliferated in some cases to the point

CHANGES IN THE INCISOR TEETH OF ALBINO RATS WITH VITAMIN A DEFICIENCY AND THE EFFECTS OF REPLACEMENT THERAPY *

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"The pathology of vitamin A deficiency indicates that the seat of the physiologic disturbances is in the epithelial cells. Chemical rôles are suppressed but proliferative powers are not inhibited; neither are the potentialities of cells lost, as is shown by the return to normal physiologic function when vitamin A is restored to the animal."—Wolbach.

The classic work of Wolbach and Howe^{1, 2} on the dental changes in vitamin A deficiency stimulated this investigation. The purposes of this study were to repeat some of their work; to study the effects of chronic vitamin A deficiency; to measure the rate of appositional growth of dentin in vitamin A deficiency; and, to interpret the findings in the light of the more recent knowledge of the histophysiology of the rat incisor.

REVIEW OF LITERATURE

The dental findings of Wolbach and Howe² may be summarized as follows: (1) The initial effect upon the incisor teeth of rats consisted of an atrophy of the enamel organ which began in the anterior portion and finally extended to the whole length of the tooth. (2) Atrophy and depolarization of the odontoblasts followed the changes in the enamel organ. The odontoblasts showed more severe alterations on the lingual side where the dentin was thin, folded, or absent. The odontoblasts survived longer on the labial side where the dentin was excessively wide. Osteoid tissue and epithelial cells derived from the enamel organ were found in the pulp. (3) Effects of replacement therapy with butter fat were noted within 7 days. Repair began in the region of Hertwig's epithelial sheath and was manifest by a recovery of the enamel organ and resumption of the normal morphology and function of the odontoblasts. Tubular predentin was now

* Aided by grants from Mead Johnson and Company and from the Carnegie Corporation of New York.

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TABLE I

Group I. Seventy White Rats Placed on a Vitamin A-Deficient Diet Arranged According to Duration of Survival Period

Sub-groups	Experimental history				Findings		
	Number of animals	Age at beginning of experiment	Age at death	Duration of experiment	Gross	Radio-graphic	Histo-pathologic
		(days)	(days)	(days)			
A	12	21	45-47	24-26	Sensitive to light; cessation in increase in weight	—	— (+)
B	17	21	55-59	34-38	Xerophthalmia; mild cessation in increase in weight	+	++
C	32	21	65-69	44-48	Severe xerophthalmia; loss in weight	++	+++
D	9	21	73	48-56	Very severe ophthalmia; loss in weight and near death	++++	++++

Number of plus (+) signs indicates degree of severity of changes.

Group II consisted of 34 rats on a vitamin A-deficient diet with replacement therapy consisting of additions of definite amounts of alfalfa or cod liver oil. Twelve animals of this group were fed a vitamin A-deficient diet for 25 days after weaning. Suboptimal rations of alfalfa were then added to the basal diet for 8 weeks. These animals survived longer than those of group I and were sacrificed at the age of 102 days (Table II). Four animals were placed on 1 per cent alfalfa-leaf meal concurrently with the basal vitamin A-deficient diet for 56 days. The remaining animals were given total replacement therapy with cod liver oil after varying periods of vitamin A deficiency.

Group III. Ninety-five albino rats were placed on a complete vitamin A-deficient diet at 21 days of age and given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S at intervals of 4 to 11 days in order to find the rate of apposition of the dentin in the incisor and of the dentin, cementum and

of replacement of the alveolar bone. Pohto¹¹ found atrophic changes in the odontoblasts that were similar to those found by Wolbach and Howe.² He emphasized the peglike dentin projections and the prominent foldings. The latter were present in the labial as well as the lingual dentin when the vitamin A deficiency was prolonged.

More recently Mellanby¹² reported on the changes in the incisors and molars of young rats whose mothers received a diet deficient in vitamin A for 5 to 7 months. The incisors showed degeneration of the enamel organ and of the ameloblasts and reduction of blood supply. Enamel was lacking in some areas. The odontoblasts degenerated on the lingual side. The dentin was poorly calcified and distorted in outline. The molars also showed defective enamel formation and poor calcification of dentin. The pulp contained ossifying areas. Mellanby emphasized the disturbance in the organizing action of the enamel organ.

For additional references the reader is referred to Wolbach and Howe² and Pohto.¹¹

MATERIAL AND METHODS

This study is based on 199 rats which were placed, at weaning, on a diet deficient in vitamin A for a period of 9 to 81 days. The animals were weaned at 21 days of age.

The diet consisted of:

Cornstarch	66.5 per cent
Casein (Vitamin A free)	18.0 per cent
Brewer's yeast	10.0 per cent
Osborne and Mendel's salt mixture	4.0 per cent
Sodium chloride	1.0 per cent
Irradiated cholesterol	0.5 per cent

The animals were weighed weekly. Vitamin A reserve was considered to be depleted when the animals became stationary in weight and showed early signs of xerophthalmia (21 to 26 days). Twelve animals of the same colony, placed on the normal stock diet, were used as controls. The experimental animals were grouped as follows:

Group I consisted of 70 rats on vitamin A-deficient diet without replacement therapy (Table I). These animals were placed on the deficient diet for a period of 26 to 56 days following weaning. They did not survive longer than 56 days.

TABLE III
*Part of Group III. Sixty Albino Rats on a Vitamin A-Deficient Diet and the Daily Rate of
 Dentin Apposition in Their Incisor Teeth*

Sub- groups	Number of animals*	Age at which alizarin red S was injected	Replacement therapy and ages at which started	Age at death (days)	Average rate of apposition per 24 hours				Approximate ratio between daily rates at midlingual and at midlabial regions
					Mid- labial (μ)	Disto- lingual (μ)	Mesio- lingual (μ)	Mid- lingual (μ)	
1	45	(days) 30, 40 43, 54 47, 57 50, 60 64, 72	None	45	16.14	14.38	13.76	13.42	3:4
			None	73	16.79	13.11	12.48	10.86	2:3
			None	77	17.91	12.85	12.13	9.57	1:2
			None	65	18.68	11.14	10.75	7.84	1:2
			None	77	19.63	11.29	9.01	6.43	1:3
2	8	47, 51	Insufficient suboptimum vitamin A replacement as blue gramma grass at 47 days	52	18.32	12.47	11.83	8.91	1:2
3	7	50, 60 60, 70	Full replacement at 50 days	75	13.12	15.78	15.76	15.81	1:1
			" " " "	75	15.97	15.83	15.80	15.91	1:1

* All rats were placed on vitamin A-deficient diet at 21 days of age (weaning).

TABLE II
Group II. Thirty-four White Rats Placed on a Vitamin A-Deficient Diet Plus a Suboptimal or Total Replacement Diet Arranged According to Duration of Survival

Sub-groups	Number of animals	Age at beginning of experiment (days)	Length of experiment prior to giving of replacement (days)	Duration of complete experiment (days)	Duration of replacement diet (days)	Type of replacement	Age at death (days)	Findings		
								Gross	Radio-graphic	Histo-pathologic
A	4	21	0	56	56	Alfalfa 1%	77	Normal
B	12	21	25	81	56	Suboptimal alfalfa	102	Xerophthalmia and loss in weight	++++	++++ No signs of repair
C	12	21	29	44-54	15-25	Total replacement with cod liver oil	65-75	Complete recovery from xerophthalmia and weight loss	+	+ Active reparative processes in proximal half of incisor
D	6	21	44	54-64	10-20	Total replacement with cod liver oil	75-85	Mild recovery from xerophthalmia and no gain in weight	++++	++++ Reparative process beginning

Number of plus (+) signs indicates degree of severity of changes

calcified in 5 per cent nitric acid for 24 hours. After dehydration and embedding in celloidin, serial midsagittal (longitudinal) and transverse sections of the incisors were stained with hematoxylin and eosin and mounted. Our studies were based mainly on longitudinal sections which facilitate the ready tracing of events from the basal to the anterior level.

The teeth of the animals that were given injections of alizarin red S were studied in ground as well as in decalcified sections. Transverse sections of upper and lower incisors were prepared by grinding on a medium and then a fine carborundum stone mounted on a dental lathe. Longitudinal ground sections of the uppers were also prepared. The lower incisors, because of the marked twist in their anteroposterior axes, were cut in half at the level of the first molar, and each half was then ground.

Measurements were made with a filar micrometer eyepiece, standardized to a stage micrometer. By measuring the distance between any two injection effects and dividing this by the time interval the daily rates of apposition were obtained.¹³ The figures on the appositional rates were subjected to statistical evaluation. Transverse and longitudinal ground sections were also prepared of the incisors of representative animals of groups I and II.

HISTOPHYSIOLOGY OF THE RAT INCISOR

Before presenting the findings in the experimental animals, we shall consider briefly those histophysiologic aspects of the incisor of the rat that have a particular bearing on the experimental changes analyzed in this report and that have become clarified during the progress of this study. The development of the incisor of the rat consists of four main stages: growth, calcification, eruption and attrition. For the purposes of this study we are primarily interested in the growth process, which passes successively through the proliferative, differentiative and appositional phases.

Odontogenic Epithelium. The rat incisor develops primarily from an elliptical epithelial base which is situated at the proximal end of the tooth and which proliferates throughout the life of the animal. Because of its function, this base may be called the odontogenic epithelium.¹⁴ It establishes the dentino-enamel and dentino-cemental junctions and thus the size and shape of the tooth. The labial third of the epithelial base overlaps a portion of the lateral surfaces (Fig. 22) and resembles in structure and in function the enamel organ of the human tooth. It establishes the dentino-enamel junction. Its inner layer, the inner enamel

TABLE IV
Part of Group III. Thirty-five Albino Rats on a Vitamin A-Deficient Diet and the Effects of Cod-Liver-Oil Replacement on the Rates of Dentin Growth in Their Incisor Teeth

Sub-groups	Number of animals*	Daily units of cod liver oil	Age at beginning of replacement (days)	Age at which alizarin was injected		Age at death (days)	Daily rates of dentin growth (μ)		Ratio of midlingual to midlabial
				1st injection (days)	2nd injection (days)		Lingual (μ)	Labial (μ)	
1	5	1	42-48	50	60	65	8.35 \pm .74	15.93 \pm .08	1:2
2	5	2	42-48	50	60	65	12.54 \pm .40	16.66 \pm .33	3:4
3	5	3	42-48	50	60	65	12.40 \pm .21	16.76 \pm .10	3:4
4	5	4	42-48	50	60	65	12.62 \pm .13	16.10 \pm .16	3:4
5	5	5	42-48	50	60	65	15.52 \pm .24	15.91 \pm .17	1:1
6	4	1	42	42	52	56	8.46 \pm .37	17.80 \pm .09	1:2
7	3	2	42	42	52	56	12.11 \pm .16	17.08 \pm .20	3:4
8	3	5	42	42	52	56	15.75 \pm .22	15.95 \pm .36	1:1

* All animals were placed on a vitamin A-deficient diet at 21 days of age and carried on the deficient diet until outward symptoms (weight gain and xerophthalmia) appeared.

alveolar bone in the molar. The animals were sacrificed from 5 to 30 days following the initial injections. Fifty animals of this group were given various levels of replacement therapy (Tables III and IV).

Radiographic and Histologic Methods

The dietary experiments and the administration of alizarin red S were carried out in the chemical laboratory at Tucson, Arizona (M. C. S.), where the gross conditions of the living animals were also observed and recorded. After death the animals were decapitated and the heads were fixed in a 4 per cent aqueous solution of formaldehyde and sent to the histologic laboratory of the College of Dentistry, University of Illinois, where the radiographic and histologic studies were carried out.

The heads were split in halves by a midsagittal cut between the left and right incisors. Each half was radiographed by exposure on a dental occlusal film for 5 seconds, $5\frac{1}{2}$ in. from the aperture of the tube casing, without the cone attached. A standard dental X-ray machine was used.

The jaws of groups I and II were washed and then de-

mately 50 days, when they become reduced and atrophied. In the adult rat (100 days or older) this state is usually reached in the anterior third of the incisor. In the young rat of 25 days of age, on the other hand, the ameloblasts maintain in the upper incisor their columnar shape even up to the gingival crest, because they require only 25 days to reach this level.

Odontoblasts and Dentin. As soon as the peripheral cells of the dental papilla differentiate into odontoblasts, they help form the dentin matrix, recede centrally, and migrate with the eruption of the tooth toward the distal end.

The pulpal recession of a given odontoblast is proportional to the amount of dentin that is laid down. The daily rate of dentin deposition is 16μ in 24 hours.¹³ The forward movement is in proportion to the rate of eruption which is about 2 mm. a week in the upper incisor and 2.8 mm. a week in the lower incisor.

In the incisor of a rat

TABLE V
Maximum Width of Dentin at Various Ages in the Incisor of the Normal Rat and that of the Vitamin A-Deficient Rat

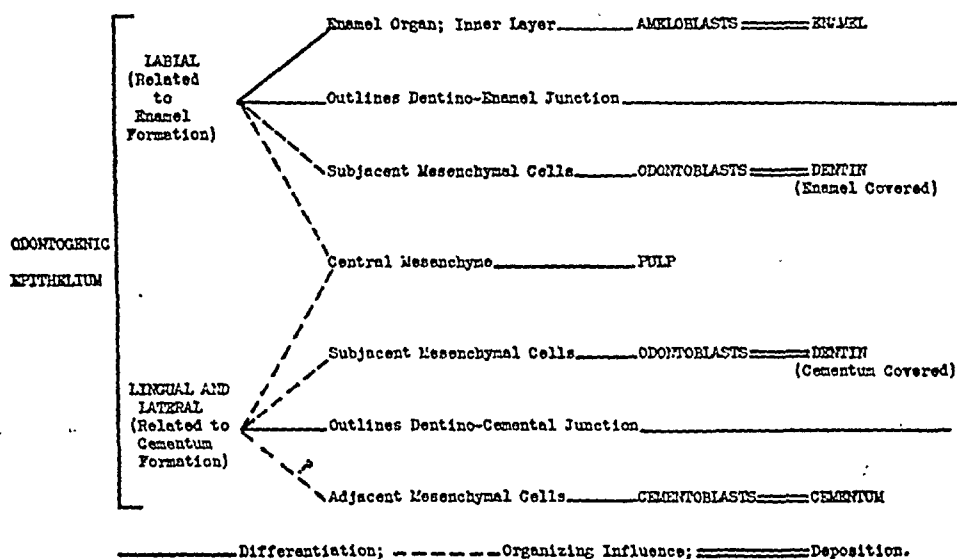
Normal controls					Vitamin A-deficient rats				
Age	Maximum width at various anatomic locations*				Maximum width at various anatomic locations†				Age‡ (days)
	Labial (μ)	Mid-lingual (μ)	Mesio-lingual (μ)	Disto-lingual (μ)	Disto-lingual (μ)	Mesio-lingual (μ)	Mid-lingual (μ)	Labial (μ)	
(days)									
58	504	489	483	497	475	416	309	511	54
60	528	505	507	508	463	421	331	531	57
70	656	587	594	610	492	430	342	658	65
77	727	634	643	678	408	374	350	744	77
100	910	849	875	887	593	541	357	1363	102

* Measurements made from transverse sections at incisal edge.

† Maximum width is attained at a more proximal level.

‡ All vitamin A deficiencies were started on the 21st day of life. Complete depletion of reserve occurs approximately 26 days later.

epithelium, differentiates into ameloblasts and, in addition, regulates and activates the subjacent mesenchymal cells facing the ameloblasts to differentiate into odontoblasts. The remaining two thirds of the odontogenic epithelium resembles in structure Hertwig's epithelial sheath and outlines the lingual and the larger portion of the lateral surfaces. It establishes the dentino-cemental junction, activates the subjacent cells of the pulp to differentiate into odontoblasts, and possibly also activates the adjacent cells of the dental follicle to differentiate into cementoblasts (Text-Fig. 1). Thus there is a striking morphologic and functional difference between the labial and lingual aspects of the odontogenic epithelium. This difference is foreshadowed even in the newborn before apposition has begun.¹⁵ For convenience we shall therefore refer to the labial odontogenic epithelium and the lingual odontogenic epithelium. Similarly, we shall distinguish between the labial or enamel-covered dentin and the latero-lingual or cementum-covered dentin, and between the corresponding labial, lateral and lingual odontoblasts.



TEXT-FIGURE 1. The histodifferentiation and organization of odontogenic epithelium.

Ameloblasts and Enamel. The ameloblasts recede peripherally from the dentino-enamel junction up to the point of maximum width of enamel, when they cease their activity in enamel apposition. The ameloblasts retain their columnar shape for approxi-

and jaws showed a reddish tint as a result of the alizarin injections.

The normal control rats showed no abnormal outward changes.

RADIOGRAPHIC FINDINGS

The normal controls showed no abnormal radiographic changes (Figs. 1 and 5).

Group I. The rats of subgroup A (Table I), which survived only 26 days, showed no abnormal radiographic changes in the incisors. The rats of subgroups B, C and D showed radiographic disturbances which progressed with the increase in survival length (Figs. 1-8).

The roentgenogram of the incisor of a normal rat presents a curved and partially hollow cylinder. The shadow is practically solid in the distal third and beginning with the middle third splits into nearly equal convex (labial) and concave (lingual) borders which taper in the proximal direction and surround the centrally located pulp (Fig. 1). In contrast, the shadow of the incisor of a rat subjected to vitamin A deficiency shows a striking distortion and gives the appearance of a sickle (Figs. 4 and 8). The convex border which represents the enamel-covered dentin is excessively wide, and at its proximal end makes a sharp bend toward the pulp. The latter is displaced toward the lingual border. The concave border, which represents the lingual dentin, is often not seen in the proximal third of the incisor and appears as a fine line only in the middle third (Figs. 4 and 8). The form of the tooth is thus distorted in a characteristic manner so that it is possible to diagnose the condition of vitamin A deficiency by an examination of the roentgenogram of the incisor of the rat (Figs. 1 to 8).

A more detailed analysis shows the following characteristic changes which permit a diagnosis of vitamin A deficiency on the basis of the roentgenogram (Figs. 2 to 4, 6 to 8):

1. The labial surface is often irregular and is foreshortened at its proximal end. Here it deflects abruptly from the normal curvature of the tooth toward the pulp.
2. The labial alveolar periosteum is widened to about three times the normal.
3. The alveolar bone at the base of the tooth tends to be more distinct and thickened.

about 100 days of age the maximum thickness of the labial dentin is found at the incisal end and is approximately $900\ \mu$ (Table V). This is associated with the fact that the life span of the odontoblasts is approximately 55 days and the daily rate of apposition is $16\ \mu$. Normally the width of the dentin, though slightly less in the cementum-covered portion, is similar throughout the circumference of the cross section of the incisor so that the ratio between the widths at the midlabial and midlingual levels is essentially 1:1.

Very little is known regarding the mechanism of the formation and calcification of enamel and dentin. There is, however, clear evidence of an intimate interrelationship between these processes. Thus normally the following orderly sequence is observed in the development of the tooth: Proliferation of the ameloblasts, differentiation of the ameloblasts, differentiation of the odontoblasts, formation of dentin matrix, formation of enamel matrix, calcification of dentin, calcification of enamel. An arrest of one step means the omission or disturbance of the subsequent processes (Text-Fig. 1).

For a detailed discussion of other phases of the normal histophysiology of the incisor of the rat the reader is referred to Schour and Steadman,¹⁴ Addison and Appleton,¹⁵ and Schour and Massler.¹⁶

RESULTS

GENERAL GROSS FINDINGS

Group I. The vitamin A body reserves were considered to be exhausted between the 24th and 26th day following the beginning of the diet. This exhaustion was indicated by cessation in gain in body weight and by slight sensitivity to light. Xerophthalmia became evident in a mild form in 34 days. Its severity gradually increased in those groups which survived 45 days or more (Table I). The gross changes in the animals of this series were similar to those reported by Smith and Lantz.⁸

Group II. Those animals which received large doses of alfalfa in their diet together with their basal vitamin A-deficient ration showed no outward changes.

Group III. The gross effects in group III were similar to those of group I. However, on dissection, the bones of the skull

Group I. Vitamin A Deficiency Without Replacement Therapy

The alterations seen in the roentgenograms were readily confirmed in the microscopic sections.

Subgroup A. Rats that Were Placed on the Experimental Diet for 24 or 26 Days Subsequent to Weaning. The newly formed lingual dentin was narrower than normal and irregular in its pulpal border. The adjacent odontoblasts were distorted. They were not columnar and had not completed their differentiation from the peripheral pulpal mesenchymal cells. The enamel epithelium was normal except for some minor hypoplastic changes in the proximal portion. The labial odontoblasts and the labial dentin were still normal. The odontogenic epithelium showed no morphologic alterations.

Subgroups B, C and D. Rats that Were Placed on the Experimental Diet for 34 to 52 Days After Weaning. The typical changes described below progressed with the increase in the survival period and varied only in degree. The disturbances were more severe in the proximal than in the distal portions (Fig. 10).

The characteristic alterations in these groups follow. Since there was a sharp difference between the changes in the enamel-covered and the cementum-covered portions of the incisor, the corresponding changes will be described separately.

*Enamel-Covered Portions of Incisor**Disturbances in Formation*

Enamel Epithelium. The epithelial papillae of the enamel organ showed occasional distortions but the changes were not prominent except in the hypoplastic areas. At the level of the alveolar crest, the papillary arrangement was, as a rule, still present. Sometimes the papillae proliferated and gathered in masses which gave the appearance of peninsulae of stratified squamous epithelium. In such cases the ameloblasts had become low or flattened or had lost their identity. Enamel hypoplasia was quite severe and common. Degenerating epithelial cells which in some instances had undergone calcification were often found in the hypoplastic crypts (Fig. 20). The ameloblasts showed premature atrophy only in the cases of longest experimental survival.

4. Enamel hypoplasia is common. In the animals with a longer survival time the proximal end of the enamel is slightly buckled and often presents a picture which simulates that of a vesicle or hollow kernel (Figs. 2, 4, 7 and 8). This differs from the usual picture of hypoplasia in that the crest of this vesicle may extend beyond the height of the enamel surface. Histologic analysis showed that these vesicles were circumscribed areas in which enamel and dentin were defective or absent and the pulp communicated with the labial alveolar periosteum through a perforation by connective tissue (Figs. 10 and 17).
5. The labial dentin is increasingly and abnormally thickened toward the distal end.
6. The pulp is thus displaced lingually.
7. The lingual dentin is very thin in the middle third and often cannot be seen in the proximal third, indicating its absence or its lack of calcification.
8. The width of the periodontal membrane is irregular and narrow, particularly at the midregion.
9. In the upper incisor the extra-alveolar portion is longer than normal and the intra-alveolar portion is shorter than normal (Figs. 1, 4 and 8). The total length of the upper incisor is, however, not longer than normal. It appears as if a portion of the tooth which is normally intra-alveolar in position has become extruded.
10. In the lower incisor the extra-alveolar portion is shorter than normal. The intra-alveolar portion usually extends proximally only as far as the mesial level of the third molar, while normally it extends beyond the third molar toward the sigmoid notch of the ramus of the mandible.
11. The incisal relationship and attrition are abnormal. The incisal bevels show a less acute angle than normal.

Group II. In the group which received minute doses of alfalfa during the last 8 weeks of life, in addition to their vitamin A-deficient basal diet, the changes were more prominent but closely paralleled the findings in subgroup D of group I (Table II, Figs. 2 and 7). Those animals which received larger doses of replacement rations in the form of 1 per cent alfalfa in their basal diet (Table II) showed no radiographic changes.

Group III. A radiographic study was not made.

HISTOLOGIC FINDINGS

Our findings confirm in the main those of Wolbach and Howe.² The emphasis in this report will therefore be placed on those findings which supplement theirs.

confined chiefly to the proximal third of the tooth. After longer survival the disturbances extended through the entire length of the lingual dentin (Fig. 10). The dentin was much narrower than normal at any particular level. Thus, at the midlingual level the width was one half the normal width or even less (Figs. 2 and 14, Table V). Its pulpal surface was irregular. The matrix frequently lacked dentinal tubules and resembled osteodentin. It contained scattered cellular inclusions and occasional vascular inclusions. The latter, however, occurred consistently near the cemento-enamel junction (Fig. 12). The odontoblasts were disorganized and showed the most severe disturbances near the proximal end. They often assumed a spheroidal outline. Osteodentin was deposited either along the lingual pulpal wall or in the pulp (Fig. 13). In the more advanced cases the dentino-cemental junction was disturbed (Fig. 12). The proximal end of the lingual dentin was situated more distally than normal (Fig. 10).

Disturbances in Calcification

The normal incremental calcification rhythm was absent (Figs. 11 and 12). The predentin was lacking and the dentin when stained with hematoxylin and eosin often took only the eosin color (Fig. 16).

Pulp. The pulp was displaced lingually, confirming the roentgenographic findings (Figs. 10 to 12). The most striking change was the invasion of epithelium which arose from the lingual odontogenic epithelium. Long cords of epithelium resembling in structure the lingual odontogenic epithelium of Hertwig's sheath extended into the pulp and continued to proliferate distally (Fig. 11). When these cords were cut transversely they gave the appearance of epithelial clusters or glandular acini. The cells were usually low cuboidal but sometimes assumed a columnar shape and arranged themselves radially (Fig. 16). The distal extent of these epithelial proliferations varied with the length of the survival. In some instances definite degenerative changes were observed which simulated thymic corpuscles (Fig. 19) and approached calcification. Osteodentin at times formed about the epithelial islands. The osteodentin was bordered by mesenchymal cells which were cuboidal in structure (Fig. 18). In longer survivals cauliflower-like islands of poorly calcified tissue projected

Enamel. The organic enamel matrix, which normally terminates at the distal end of the proximal third of the tooth (about 7.5 mm. from the odontogenic base), was found to terminate between 50 and 200 μ from the odontogenic base (Figs. 9 and 10). In the longer survivals the organic enamel matrix and the corresponding dentin were often buckled and wavy in the extreme proximal portion (Fig. 10).

This picture reminds one of that seen with long survival after hypophysectomy.¹⁷ In hypophysectomy, however, the foldings were deeper and more numerous. Often, concurrently with these violent disturbances, a large vesicular area was observed near the proximal end of the organic enamel matrix (Figs. 10 and 17). This was the vesicle described in the X-ray findings. It communicated with the pulp and was lined peripherally with normal odontoblasts. The lumen was filled with pulpal cells and an occasional island of epithelium and osteodentin.

Labial Dentin. This dentin was wider than normal (Table V), but for the most part normal in structure and in staining reaction. In the proximal region the odontoblasts as a rule showed no morphological disturbances. In the distal region they attained a cuboidal and finally a spheroidal form and resembled osteoblasts. They appeared to have lost their attachment to the dentin and to have migrated toward the center of the pulp. Here, islands of osteodentin with cellular and vascular inclusions were abundant. In some of the animals of longest survival the odontoblasts showed alterations even in the proximal region.

Disturbances in Calcification

In some animals the dentin showed to a marked degree interglobular dentin which was accompanied by an abnormal width of predentin (35 to 50 μ) at the midthird level. It was found that these animals had received a basal diet which was less fortified with vitamin D than usual. These animals showed in addition fibrotic changes in the pulp that resembled scar tissue and that were more prominent than those found in the other experimental animals (Fig. 26).

Cementum-Covered Portion of the Incisor

Disturbances in Formation

Dentin. The normal histologic characteristics of the lingual dentin were lost. In animals of shorter survival the changes were

were prominent and very long, with the cementing lines staining readily with hematoxylin. The spicules were arranged regularly and parallel with the long axis of the incisor.

Transverse Sections. In cross sections two prominent and characteristic vascular inclusions extended along the dentinal tubules and coursed from the mesial and distal cemento-enamel junctions to the pulpal wall. It is interesting to note that the greatest disturbances of the tooth were located lingual to these two outstanding vascular inclusions (Fig. 12).

Transverse sections also indicated clearly the extreme distortion of the growth pattern that is characteristic of vitamin A deficiency. The pulpal surface which normally parallels closely the outline of the dentino-enamel and dentino-cemental junctions showed no order or regularity (Fig. 12). The dentino-cemental surface was also distorted and occasionally in localized areas the pulp and periodontal membrane communicated. In these areas fibrous bands of connective tissue tended to bridge the perforation and wall off and separate the pulp from the periodontal membrane. The cementum adjacent to the perforated areas was thickened (Fig. 12).

Group II. Vitamin A Deficiency with Replacement Therapy

Subgroup A. These animals which were given total replacement with 1 per cent alfalfa concurrently with the basal diet showed no abnormal changes in the incisors (Table II).

Subgroup B. The animals in subgroup B which were given replacement with a suboptimal amount of alfalfa during the last 8 weeks, following a period of complete vitamin A deficiency, showed histopathological disturbances which were similar to those observed in subgroups B, C and D of group I. The premature atrophic changes in the enamel organ were more evident in this group than in group I, probably because of the longer survival period.

Subgroups C and D. The teeth of those animals which were given full replacement therapy for 10 to 20 days showed evidence of a resumption of normal histodifferentiation. The peripheral mesenchymal cells of the pulp, which are responsible for dentinogenesis, and the new matrix in the cementum-covered dentin, which appeared since the institution of replacement and

consistently from the lingual dentin into the pulp. They reminded one of the osteoid proliferations seen in rachitic compensatory hyperplasia. Encircling these islands and often caught in the meshwork of the matrix were found hematoxylin-staining cells which were spheroidal in shape and which differed markedly from the normal columnar-shaped odontoblasts (Figs. 11 and 13). The pulpal wall was very irregular and bayed as a result of the incomplete fusion of these islands. From these bays and irregularities many vascular inclusions dipped inward and penetrated the dentin for quite a distance. These inclusions often branched within the dentin matrix (Fig. 12).

The blood supply of the pulp was prominently reduced on the lingual surface. The connective tissue of the pulp tended to lose its normal embryonic-like character and became more fibrous, especially near the epithelial cords (Fig. 26). Calcospherites were not common.

Cementum. The cementum, which normally assumes a maximum width of 3 to 4 μ , often approached a width of 10 to 15 μ . The staining reaction with hematoxylin was pale. In occasional areas of communication between the pulp and the periodontal membrane the adjacent cementum was excessively wide, as if to compensate for the lack of attachment and cementum at the points of communication. Here, in place of the cementum, fibrous condensations of connective tissue tended to bridge the gap (Fig. 12).

Periodontal Membrane. In the normal rat the outline of the periodontal membrane, both on the cementum and the alveolar side, is fairly regular. Its width varies between 240 and 275 μ in the proximal half and between 100 and 125 μ in the distal half. In vitamin A deficiency the outline of the periodontal membrane was very irregular. The width varied considerably (40 to 200 μ) and in general was narrower than normal (Figs. 9 and 10).

Labial Alveolar Periosteum. The labial alveolar periosteum was considerably widened. It contained subacute inflammatory cells, crystals and fibrinous strands similar to those seen in clotting blood (Fig. 13). With late survival the blood supply of the periosteum seemed to be reduced.

Alveolar Bone. The socket bone (surrounding proximal base) appeared to be thicker than normal. The spicules in the bone

posed at the time of the injections. The rings were more intensely stained, sharp and distinct in the enamel-covered dentin, but wavy and relatively faint in the cementum-covered dentin. The course of the alizarin rings, in cross section, paralleled the outline of the pulpal wall and showed indentations with each vascular inclusion. The distance between the effects of any two injections on the labial aspect, from the cemento-enamel-junction level on the mesial to the corresponding level on the distal portion, was fairly parallel. The distance between the two lines was found to be much smaller immediately lingual to the cemento-enamel junctions, and sharply diminished toward the midlingual region of the tooth, where the distance between the two rings and the entire width of the dentin was narrowest. (Text-Fig. 2, Figs. 22 and 23).

The daily rate of apposition of both the enamel-covered and cementum-covered dentin was found, on the basis of more than 200 measurements on 12 control animals, to range between $15.25 \pm 0.51 \mu$ and $16.12 \pm 0.73 \mu$. These figures were not significantly different from the normal average daily appositional rate of 16μ found in the dentin of normal rats in previous reports.¹³ The differences in the normal daily rates in the labial and lingual portions are very slight, so that the ratio of the thickness of the midlabial and midlingual dentin is 1:1.

The disturbances in the rate of appositional growth were manifest between 9 to 19 days following institution of vitamin A deficiency, long before the cytologic and histologic changes become apparent (Table III). The daily rate of apposition of enamel-covered dentin was found to be greater than normal and increased up to 19.63μ , as the duration of vitamin A deficiency increased (Table III, Text-Fig. 2). The daily rate of apposition of cementum-covered dentin was less than normal and decreased as the survival period increased. In addition to this survival or age gradient, apposition also followed a locus gradient which decelerated uniformly from the cemento-enamel junction where it was relatively highest to the midlingual level where it was lowest (6.43μ) (Table III).

Suboptimal replacement therapy of 4 days' duration did not produce measurable changes in appositional rates. However, full replacement therapy had an immediate effect, causing the normal

which is found first at the proximal base of the tooth, were normal. The changes which had occurred prior to replacement therapy were still present in the middle and distal thirds of the tooth. These areas, however, showed a greater tendency toward fibrosis of the injured or malformed tissues than in animals at deficiency levels.

In the 10-day-replacement animals a spearlike projection of dentin extended obliquely from the linguo-proximal base into the pulp. This projection of dentin was bordered on the pulpal surface by normal odontoblasts. The dentin and predentin were of normal texture and showed a normal staining reaction (Fig. 24). The width of the dentin was $110\ \mu$. Assuming that it was apposed at the normal daily rate of $16\ \mu$, it was estimated that the new dentin had been apposed for approximately 7 days before death. In other words, apposition of new dentin had begun within the third day of the replacement period.

The form of the ectopic dentin laid down during early replacement depends upon the arrangement of the epithelial islands that had invaded the pulp during the deficiency. In the case of a spearlike epithelial invagination (Fig. 25) the newly formed dentin assumed a spearlike pattern (Fig. 24). Transverse sections of the newly formed dentin showed isolated circular areas of dentin with epithelial cells in the center (Fig. 18). These cells evidently regained their capacity to cause adjacent cells to differentiate into odontoblasts but did not acquire any amelogenic capacity. In no case was enamel or enamel-like tissue found in the pulp.

The changes during the 15 to 20 day repair period were similar to those for the 10 day period but more advanced.

Group III. The Effects of Vitamin A Deficiency Studied by Means of Vital Staining with Alizarin Red S

The data for this group were obtained from ground sections which were necessary for the determination of the rate of apposition since the alizarin effects were lost during decalcification. The findings, other than those due to alizarin, were those characteristic for the corresponding degree and duration of vitamin A deficiency.

Injections of alizarin red S produced red lines which were superposed on the daily incremental rings which were being ap-

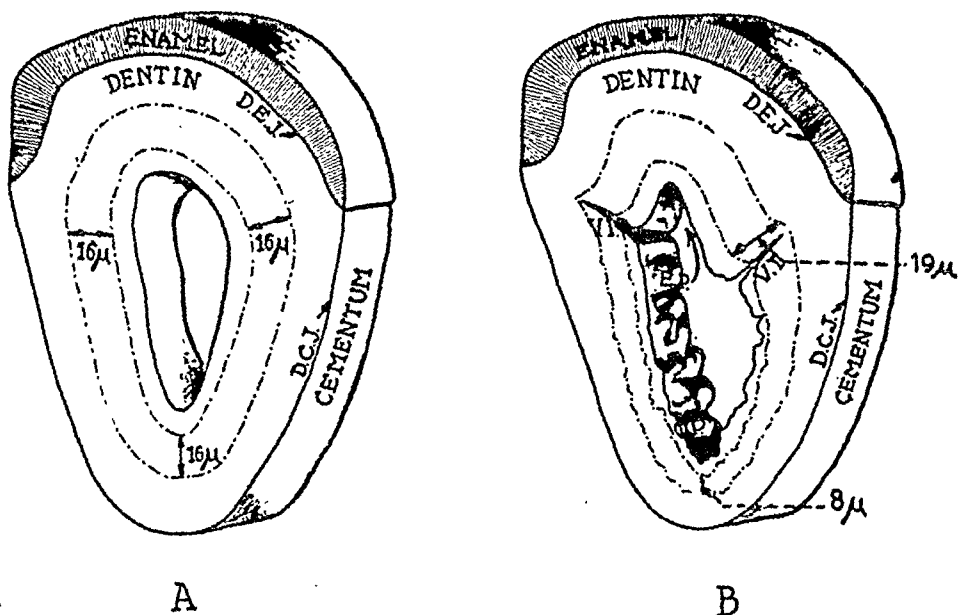
of the dentin were thus due primarily to the differences in the rate of growth, and the ratio between the total thicknesses of the midlingual and midlabial dentin may be used as an index of the severity of vitamin A deficiency. The longer the duration of vitamin A deficiency, the greater the ratio between the daily rates or total dentin thickness of the midlingual and midlabial levels. Thus in the normal control, or in full replacement, the ratio is 1:1. During the second and third weeks following the institution of a vitamin A-deficient diet the ratio was 3:4; during the fifth and sixth weeks the ratio was 1:2, and during the seventh week, 1:3 (Table III).

Summary of the Effect of Vitamin A Deficiency on the Rate of Dentin Apposition. The rate of apposition of dentin was selectively altered in vitamin A deficiency, while the life span of the formative cells was not affected. The rate of apposition was accelerated in the enamel-covered portion and decelerated in the cementum-covered portion, with a uniform gradient effect. The extent of the deviation from the normal was in direct proportion to the duration of the vitamin A deficiency and may be expressed in the ratio between the total dentin width or daily rates at the midlingual and midlabial levels.

The Rate of Apposition of Dentin in Vitamin A Deficiency Followed by Graded Replacements. Table IV gives the experimental history and the quantitative findings in animals which were given graded daily replacement doses of cod liver oil following the depletion of the vitamin A reserves. One Sherman unit of vitamin A fed as cod liver oil did not measurably affect the rate of growth of dentin over that of the totally deficient animals (Table III). Two to four units, inclusive, had the effect of reestablishing the labial rates to approximately normal. However, the lingual rates, though higher than in total deficiency, remained at a ratio of 3:4 to the labial rates up to the 23rd day following the institution of replacement. The five-unit replacement showed a resumption of normal rates of growth on both the labial and the lingual dentin by the tenth day following the beginning of replacement therapy.

FINDINGS IN MOLAR TEETH

Histologic Changes. The molar changes resulting from vitamin A deficiency were in direct proportion to the chronologic stage of



TEXT-FIGURE 2

(From Schour, Smith, and Hoffman: *Proc. Soc. Exper. Biol. & Med.*, 1938, 39, 448)

A. Microprojector tracing of section shown in Figure 22 from a normal control rat which was given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S on the 50th and 60th days of life and sacrificed on the 65th day. Note that the alizarin red S effects (dotted lines) are parallel. The outline of the pulpal wall is regular, smooth and closely parallels the outline of the dentino-enamel (D.E.J.) and dentino-cemental (D.C.J.) junctions. The rate of apposition approximates $16\ \mu$ per day. $\times 71$.

B. Microprojector tracing of section shown in Figure 23 of a vitamin A-deficient rat which was put on the deficiency diet on the 21st day of life; given intraperitoneal injections of 0.5 cc. of a 2 per cent solution of alizarin red S on the 50th and 60th days and sacrificed on the 65th day. Contrast with A and note (1) the greater thickness of the enamel-covered dentin; (2) the narrowness of the cementum-covered dentin; (3) the irregular and distorted pulpal outline with the deep vascular inclusions, V.I., at the cemento-enamel junctions, C.E.J. The pulpal space, E.P., which is next to the enamel-covered dentin, is considerably narrowed in contrast to the pulpal space, C.P., which is next to the cementum-covered dentin. The alizarin red S effects are indicative of a marked gradient resulting from the increased daily rate of apposition in the enamel-covered dentin ($19\ \mu$) and the decreased rate of apposition on the cementum-covered dentin ($8\ \mu$). $\times 71$.

rate of dentin apposition (approximately $16\ \mu$ per 24 hours) to be resumed within a period of 1 to 5 days.

By measuring the total width of dentin at various anatomic locations (Table V) and dividing it by the estimated average daily rate of apposition it was found that while the rate of apposition in rats on vitamin A deficiency differed markedly from the normal, the life span of the formative cells showed little if any differences from the normal. The differences in the total width

essential sequences of events and the interesting interplay between the remarkable organizing influence of the odontogenic epithelium and the responsive pulpal mesenchyme. This reconstruction is given in Text-Figure 1 and facilitates an understanding of the sequence of events in vitamin A deficiency.

THE EFFECTS OF VITAMIN A DEFICIENCY ON HISTODIFFERENTIATION

The characteristic dental changes point to the view that in vitamin A deficiency the primary and basic alteration lies in a disturbance of the odontogenic epithelium and specifically in the process of histodifferentiation of these cells. This interpretation is in accord with the view taken by Wolbach and Howe² and more recently by Wolbach.³ Most of the other dental changes, such as the uninhibited proliferative growth of the odontogenic epithelium and the disturbances in appositional growth, may be regarded as secondary effects which are the resultants of a disturbance in histodifferentiation. In vitamin A deficiency the effect on histodifferentiation is evidenced by the following:

TABLE VI
*Measurements of Dentin, Cementum and Alveolar Bone Growth, and Rates of Eruption of First Molar for a Period of 50 to 65 Days in 10 White Rats Placed on Vitamin A Deficiency**

	Rates of apposition per 24 hours										Widths				Rates of eruption per 24 hours
	Dentin—tooth levels				Secondary cementum	Alveolar bone		Periodontal membrane					Secondary cementum		
	Mid-crown	Cemento Enamel junction	Mid-root	Apical third		Fundus	Crest	Root level in thirds				Fundus			
								Cervical	Middle	Apical					
Vitamin A deficient	4.2	3.6	1.7	1.3	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	(μ)	14.8		
Normal	5.2	4.5	2.8	2.4	9.8	7.2	11.4	52	58	104	125-130	52	19.7		
					12.4	7.0	102	118	131	122-129	174				

* These animals were placed on vitamin A deficiency at 21 days of age, injected with alizarin red S on the 50th and 60th days and sacrificed on the 65th day of age.

development of the tooth at the period when the vitamin A reserves were depleted in the animal. Since in the experimental animals the vitamin A body reserves were not exhausted until about the 45th day when the crowns and a considerable portion of the roots in the molar had completed their formation, the changes were slight in contrast to those observed by Mellanby,¹² in whose animals vitamin A deficiency was instituted in the maternal diet.

In our material the third molar, which is the last tooth to develop, showed a very irregular pulpal outline in the root portion. The odontoblasts were scattered or absent in localized areas. Projections of osteodentin were seen to grow out into pulp at various levels of the root. Epithelial islands were found near the pulp chamber and in the middle third of the root. Cysts were noted in several instances. In the first and second molars an abnormal number of epithelial pearls were present in the apical third of the roots (Fig. 27).

In animals of longer survival period (chronic deficiency) the changes were more severe. The dentin of the apical third as well as the last formed secondary dentin were irregular in their outline and amorphous in character.

Appositional Rates of Cementum and Alveolar Bone. Injections of alizarin red S produced in the first molar sharp, continuous red lines in the coronal and middle thirds, but distorted, irregular and discontinuous lines in the apical third. The irregularity of the alizarin lines in the secondary cementum similarly indicated an abnormal, amorphous formation. Table VI gives measurements of the daily rates of apposition of dentin, cementum and alveolar bone of the first molar. The rates were significantly lower than those in the molar of the normal rat.^{18, 19} The width of the periodontal membrane was narrower than normal except for the fundic portion. The rate of eruption was retarded.

DISCUSSION

Interrelationship Between the Odontogenic Epithelium and the Pulpal Mesenchyme. An analysis of the dental effects of vitamin A deficiency supported by recent studies in tissue culture²⁰ and transplant experiments²¹ has thrown valuable light on the physiologic interrelationships and interdependencies of the various phases of tooth development. We may now reconstruct the

portion of the odontogenic epithelium. In the latter, the capacity of the inner enamel epithelium to differentiate into ameloblasts is less severely disturbed.

Consideration of the Differences in the Reaction of the Labial and Lingual Dentin. The current knowledge of the normal embryologic and morphologic differences between the labial and lingual portions is not sufficient to explain the selective difference in the effects of vitamin A deficiency on the morphologic alterations and the rate of apposition. Wolbach and Howe² (1933) attributed to the enamel and the enamel organ a protective chemical function for the underlying labial dentin and its odontoblasts. While the decreased rate of apposition of the cementum-covered dentin is in harmony with the decreased body weight in vitamin A deficiency, the accelerated appositional rate of the enamel-covered dentin cannot be readily explained. A teleological consideration suggests that in the presence of a severe disturbance there is a compensatory thickening of the labial dentin which has to carry most of the functional stress in mastication.

Disturbances in Odontoblasts not Merely an Atrophy but a Lack of Normal Differentiation. The fact that in a given case the disturbances in the odontoblasts were more severe in the proximal than in the distal portions may be explained on the basis that the proximal tissue having been formed more recently was subject to the state of more advanced deficiency, associated with the longer survival period. It gives support to the interpretation that the odontoblasts are disturbed not because they have a lack of proper nutrition and thus atrophy, but rather because initially they are not given the opportunity to differentiate properly. The presence of normal odontoblasts in replacement therapy does not necessarily represent a recovery of atrophied odontoblasts. They rather appear to be new and young odontoblasts that have become differentiated from the mesenchymal cells under the influence of the now normally functioning odontogenic epithelium.

Disturbances in the Proliferative Growth of the Odontogenic Epithelium. Normally the proliferative phase of cellular growth becomes limited or ceases upon the assumption of histodifferentiation. It appears that in vitamin A deficiency the proliferative phase of growth of the cells of the odontogenic epithelium

1. Proliferative growth does not cease completely. Epithelial cells proliferate into the pulp. Wolbach and Howe² recognized the "acquisition of the neoplastic properties." The findings of Orten, Burn and Smith¹⁰ of odontomas in prolonged chronic vitamin A deficiency may thus be explained.
2. The morphologic plan is disturbed. The outline of the dentino-enamel and dentino-cemental junction is distorted and often dysplastic.
3. The morphologic differentiation of the lingual odontogenic epithelium is incomplete.
4. The organizing influence of this epithelium upon the subjacent mesenchyme is thus incomplete.
5. The daily rate of dentin apposition is subsequently altered (increased rate on the labial and decreased rate on the lingual), although the life span of the cells appears to remain normal.

These changes represent experimentally induced accentuations or alterations of the following phases which normally occur in and closely follow histodifferentiation:²²

1. Proliferation ceases. Histodifferentiation marks the end of the proliferative phase of cellular activity.
2. Establishment of the morphologic plan (the dentino-enamel and dentino-cemental junctions).
3. Morphologic differentiation of the cells (differentiation of cells of the inner enamel epithelium into ameloblasts).
4. Organization of adjacent mesenchymal cells by the epithelium.
5. Preparation for apposition which normally proceeds at a definite daily rate of activity during the life span of the cell.

The earliest specific effects upon the odontogenic epithelium are first recognized not in morphologic alterations but in functional behavior. The various functions of the odontogenic epithelium are not disturbed equally. The earliest effect is manifested in the linguo-lateral portion of the odontogenic epithelium which lacks the organizing principle that normally enables it to guide, stimulate and "organize" the mesenchymal cells of the pulp to differentiate into active dentin-forming cells. This capacity to organize mesenchyme is apparently much more severely disturbed in the lingual and latero-lingual portions than in the labial

ance than the odontoblasts. Premature atrophy of ameloblasts which, according to Wolbach and Howe,² represents the earliest response, was not observed in animals that were placed on vitamin deficiency for less than 60 days. Within this period we were unable to observe any deviation from the normal process. The rate of progress of cytomorphosis in the incisor of the adult rat is such that during normal eruption the normal atrophy and retrogression begin when the ameloblast has reached the incisal third of the tooth.²³ Premature retrogression of the ameloblasts was observed in animals that were on vitamin A deficiency for 82 days. This change, however, is not specific for vitamin A deficiency. It is readily found in vitamin B deficiency,²³ hypophysectomy,¹⁷ parathyroidectomy²⁴ and magnesium deficiency.²⁵ Atrophy may also be found in a relatively more proximal position than normal in cases of retarded eruption. Here the ameloblasts will reach their stage of atrophy within the same approximate 50-day period, but their position in the anteroposterior direction, which is determined by the eruption rate, will be relatively more proximal than normal. In accelerated eruption, such as can be produced by cutting off the exposed portion of the incisors, the atrophy of the ameloblasts is not reached and their tall columnar appearance is retained even at the gingival crest.²⁶

Vascular Inclusions. The vascular inclusions in vitamin A deficiency are characteristic and differ from those seen in parathyroidectomy.²⁴ In the latter condition the vascular inclusions recur at more or less regular intervals and penetrate the dentin in an almost straight line. The vascular inclusions in vitamin A deficiency are more numerous and branch within the matrix of the dentin.

In addition, especially deep vascular inclusions occur consistently at the cemento-enamel junction and represent a most severe alteration in dentin formation. In vitamin A deficiency the cemento-enamel junctions are critical sites where the growth gradients change suddenly from an increased daily rate above $16\ \mu$ to a decreased daily rate below $16\ \mu$. The vascular inclusion appears to be a secondary effect following the premature cessation of odontoblastic growth.

To our knowledge, vascular inclusions in the human teeth at the cemento-enamel junction have not been reported. However,

is unchecked in proportion to their inability to reach the subsequent differentiative phase of growth. The lingual odontogenic epithelium shows prominent proliferation and invasion into the pulp. On the other hand, the labial odontogenic epithelium is much less disturbed in its histodifferentiation than is the lingual portion, and also shows relatively less proliferation into the pulp.

The Behavior and Fate of the Epithelial Cells in the Pulp. The continually proliferating epithelium appears to survive readily in the nutritive connective tissue of the pulp. Here it does not become a stratified squamous type of epithelium and shows no tendency to keratinization or cyst formation. On the other hand, there is a characteristic tubular arrangement which is suggestive of a glandular organ and which reminds one of the epithelial cords and rests observed normally in the periodontal membrane. Some of the proliferating epithelium, however, still possesses the chemotactic property to stimulate dentin formation. But this capacity is weak and defective so that the result is not a true dentin product but an irregular, amorphous and unorganized matrix.

In replacement therapy the organizing influence of the epithelium is regained. The adjacent mesenchymal cells become differentiated into odontoblasts and normal dentin is apposed within the pulp. The amelogenic capacity, in contrast, is not manifested even after replacement. The reason for this is probably the fact that the invading epithelium is derived chiefly if not entirely from the lingual odontogenic epithelium which even normally does not possess amelogenic capacity.

The amount of pulpal epithelium increases with the duration of the survival period. In chronic vitamin A deficiency extending over very long periods¹⁰ the epithelium is found in the adjacent tissues. Signs of degeneration of the pulpal epithelium are not frequent although sometimes areas of hyalinization or nests that resemble Hassall's corpuscles (Fig. 19) are seen. The absence of epithelial cyst formation in the pulp and its presence in the periodontal tissue is also interesting. Huggins, McCarroll and Dahlberg²¹ found that when isolated enamel epithelium was transplanted the epithelial cells did not become cystic but formed islands and cords of cells (with epithelial pearl formation).

Atrophy of Ameloblasts. The ameloblasts show less disturb-

SUMMARY AND CONCLUSIONS

The effect of vitamin A deficiency upon the development of the incisor and molar teeth of the white rat was studied in 199 animals, in respect to alterations seen in roentgenograms and in histologic sections. Eighty-four of these animals were given various types of replacement therapy; 95 animals were subjected to vital staining with alizarin red S in order to study the rates of apposition of dentin.

The characteristic roentgenologic changes are described.

The histophysiologic findings were:

1. The primary effect of vitamin A deficiency is on the histodifferentiation of the odontogenic epithelium.
2. Histodifferentiation, particularly of the lingual odontogenic epithelium, is disturbed and incomplete, with the result that its normal *organizing influence* causing the pulpal cells to differentiate into odontoblasts is ineffective. The earliest response can be recognized in a morphologic and functional alteration of the lingual odontoblasts rather than in any morphologic change of the epithelium itself. The lingual dentin is abnormally thin.
3. Concomitant with the lack of histodifferentiation there is a continuation of the proliferative activity of the odontogenic epithelium. The result is an invasion of the pulp by epithelial cords which arise for the most part from the lingual odontogenic epithelium.
4. The morphologic outline of the tooth is distorted.
5. The rate of dentin apposition is selectively altered. The enamel-covered dentin shows an accelerated and the cementum-covered dentin a decelerated rate of apposition.
6. The pulpal epithelium has an aberrant organizing influence upon the adjacent mesenchyme which forms amorphous dentin.
7. Replacement therapy results in the resumption of the normal rate of dentin apposition and the prompt differentiation of the peripheral pulpal cells into odontoblasts.

it is not rare in human teeth to observe a vascular inclusion extending from the growth center to the pulpal horn. The incidence in human teeth and in the molars of the rat of pulpal inclusions below the growth centers may be explained on the basis of greater mechanical crowding of the formative cells with the result that some of them undergo atrophy and become embedded. It is possible that in the rat incisor the characteristic vascular inclusions at the cemento-enamel junction may also be due in part to a greater crowding of the odontoblasts. The cemento-enamel junction as seen in cross sections takes the form of an indentation. The change in the curvature of the dentinal tubules in this area gives further indication of the crowding of the odontoblasts.

The Effects of Replacement Therapy on the Rate of Apposition of Dentin. The findings demonstrate that dentin apposition, which was among the first processes to show manifestation of vitamin A deficiency, was also among the first to respond to replacement therapy. Thus dentin is not only a delicate recorder of alterations in calcium metabolism^{27, 28} but also acts as a growth kymograph.

The findings in Table III show a selective response of the rate of dentin apposition to different degrees of deficiency (on the basis of the survival periods). These findings thus first suggested the possible use of this reaction as a biological method of measuring vitamin A content in foods. To test this possibility, a group of 35 rats was placed on total vitamin A deficiency and then given graded doses of replacement therapy (Table IV). The results showed significant differences in response to the daily administrations of 1 and 5 units of cod liver oil but showed no significant selective response to doses of 2 to 4 units.

It is evident that any attempt to utilize total vitamin A-deficient animals for a quantitative assay method for vitamin A content in foods would not be satisfactory. However, it is possible that a careful quantitative biologic assay for vitamin A content in foods may prove successful if normal animals, which would be put on a vitamin A-deficient basal diet plus graded doses of replacement, were used. It would then be of interest to see whether graded doses produce correlative gradients in appositional growth of dentin.

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Our findings confirm on the whole those of Wolbach and Howe,² although the duration and composition of our experimental diet was different.

The reaction in vitamin A deficiency offers ideal material for the analysis of a number of physiologic processes in tooth development.

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DESCRIPTION OF PLATES

PLATE 100

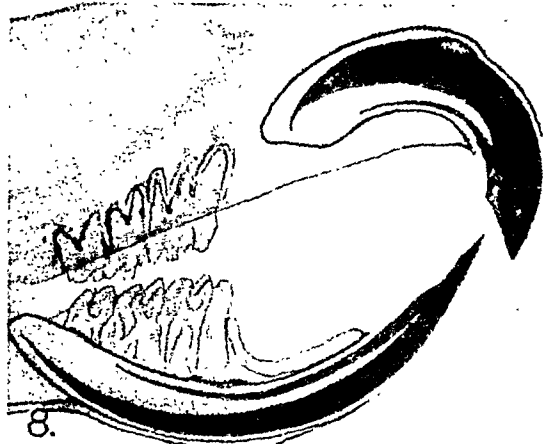
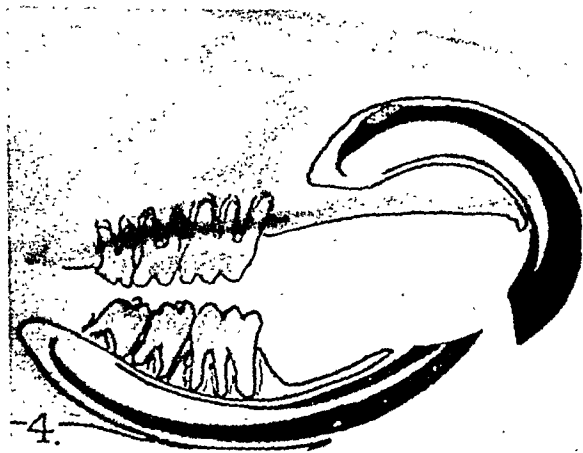
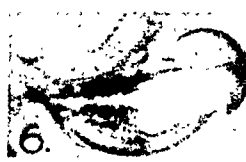
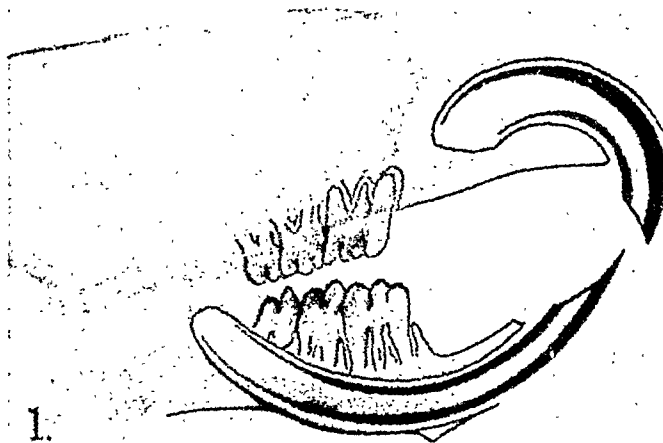
- FIG. 1. Roentgenograms of right half of head of a normal control rat 43 days old. The calcified portions are traced in black. Compare with Figures 4 and 8. $\times 2.75$.
- FIG. 2. Roentgenogram of right half of head of an albino rat which was on a vitamin A-deficient diet for 81 days following weaning and was given 0.0075 gm. of alfalfa daily for the last 56 days. Note extreme thinning of lingual dentin; increased thickness of labial dentin; increased extra-alveolar length of the incisors; wide labial alveolar periosteum especially at basal zone; the sharp bend, pulpally, of the proximo-labial base; bleb on the labial surface of the upper incisor; dulled incisal bevels. Compare with Figures 4 and 8. Natural size.
- FIG. 3. Roentgenogram of right half of head of a rat which was on a vitamin A-deficient ration for 49 days after weaning. Note position of the bleb in proximal zone and compare with Figures 2, 7 and 8 in which the survival was 81 days. The blebs in the longer survivals are located further distally. Natural size. (See Fig. 10.)
- FIG. 4. Enlargement of roentgenogram of Figure 3 in which the calcified dental structures are traced in black. $\times 2.75$.
- FIG. 5. Roentgenogram of right half of head of a rat which was on vitamin A-deficient ration for 56 days, but was given an optimum daily replacement in the form of alfalfa. Picture of roentgenogram is normal. Compare with Figure 1, and contrast with the other figures. Natural size.
- FIG. 6. Roentgenogram of right half of head of a rat which was on a vitamin A-deficient ration for 52 days following weaning. Note characteristic changes referred to in Figure 2. Natural size.
- FIG. 7. Roentgenogram of right half of head of a rat which had a similar history as the animal in Figure 2. Compare with Figure 8. Natural size.
- FIG. 8. Enlargement of roentgenogram of Figure 7, showing semidiagrammatic sketch of the incisor teeth. Note abnormal curvature and characteristic changes referred to in Figure 2. Compare with Figure 1. $\times 2.75$.

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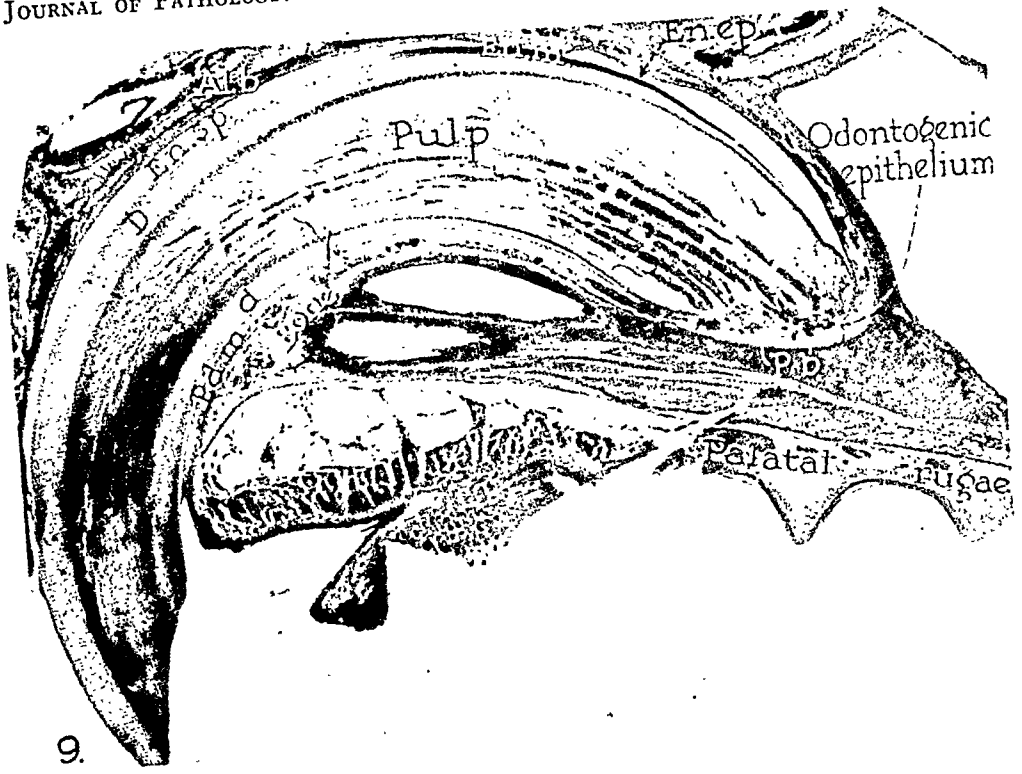
PLATE 101

FIG. 9. A midsagittal section of an upper incisor of a normal rat 65 days old. Note the extension of the pulp to the distal edge and the relative thickness of the labial, D, to the lingual, d, dentin. Al.b. = alveolar bone; D = dentin; En.ep. = enamel epithelium; En.m. = organic enamel matrix; En.sp. = enamel space formerly occupied by enamel lost in decalcification; P.b. = proximal base of alveolar bone; P.d.m. = periodontal membrane. $\times 11$.

FIG. 10. A midsagittal section of an upper incisor of an albino rat, 70 days old, which was on a vitamin A-deficient ration for 49 days after weaning. Note the relative thickness of the labial, D, to the lingual, d, dentin. En.hy. = enamel hypoplasia; En.m. = shortened organic enamel matrix; P.b. = thickened proximal bone; P.d.m. = narrowed periodontal membrane; V = vesicle on labial surface, characteristic for vitamin A deficiency. See Figures 3 and 4. $\times 11$.



- FIG. 11. A midsagittal section of the upper incisor of an albino rat which was on the vitamin A-deficient diet for 81 days after weaning and was given a suboptimum dosage of alfalfa (0.0075 gm. daily) after the vitamin A reserve was depleted (25 days following the beginning of the deficiency diet). The animal was sacrificed at the age of 102 days. En.m. = abnormally shortened organic enamel matrix; En.ep. = enamel epithelium; En.sp. = enamel space; En.hy. = enamel hypoplasia; Ep.i. = epithelial islands which arose from the odontogenic epithelium, od.ep., and proliferated in pulp; L.a.b. = labial alveolar bone; D = interglobular and thickened enamel-covered dentin; L.a.p. = abnormally widened space of proximal labial alveolar periosteum; d = narrowing and abnormal morphology in the cementum-covered dentin; Od. = odontoblasts (lost on lingual); Osd. = osteodentin on lingual wall; P.osd. = peninsula of osteodentin extending into pulp. $\times 20$.
- FIG. 12. A cross section from the proximal third of the lower incisor of a rat which had the same experimental history as the animal in Figure 11. Note the prominent distortion of the tooth and the marked thickening and interglobular nature of the enamel-covered dentin, D. O and O' indicate absence of cementum-covered dentin. Here the pulp communicates with the periodontal membrane, P.d.m., which is abnormally wide at the lingual aspect; v.i. = deep vascular inclusion which runs to the cemento-enamel junction, C.e.j.; Al.b. = alveolar bone; En.sp. = enamel space; d = atypical cementum-covered dentin; y = hypercementosis. $\times 45$.
- FIG. 13. A longitudinal section of the upper incisor of a rat which was on the vitamin A-deficient diet for 52 days after weaning. The animal was sacrificed at the age of 73 days. Note the thickened enamel-covered dentin, D. The organic enamel matrix, En.m., is shorter than normal and shows a definite area of hypoplasia, En.hy. At this level there is no dentin on the lingual to correspond with the formed labial dentin. Instead, a large accumulation of osteodentin, Ost.d., can be seen. (En.ep. = enamel epithelium which is still active in middle third of tooth; En.m. = abnormally shortened enamel matrix; L.a.p. = widened proximal area of labial alveolar periosteum containing large tissue spaces; Od. = odontoblasts of the labial pulpal wall.) No odontoblasts are seen on the lingual wall. $\times 30$.
- FIG. 14. A midsagittal section of the middle third of the upper incisor of a rat which was on a vitamin A-deficient diet for 50 days after weaning. Note interglobular and widened enamel-covered dentin, D; narrowed lingual dentin, d. (contrast with FIG. 15); islands of osteodentin, Ost.d., near lingual wall; epithelial islands, Ep.isl., in pulp near lingual wall; Od., labial odontoblasts. There is no evidence of lingual odontoblasts. Al.b. = alveolar bone; En.ep. = enamel epithelium; En.sp. = enamel space; P.d.m. = abnormally narrowed periodontal membrane for this level. $\times 45$. Contrast with Figure 15.
- FIG. 15. A midsagittal section of the upper incisor of a normal rat illustrating normal cementum-covered dentin, d., and normal periodontal width, P.d.m. Note presence of odontoblasts which are lacking or deficient on the lingual wall in vitamin A deficiency. Contrast these conditions with Figure 14. $\times 90$.
- FIG. 16. A midsagittal section of the upper incisor of a rat with similar history as that used for Figure 12, showing high power field of the proliferating epithelial islands, Ep.i., in the pulp similar to those seen in Figure 11. Abnormal lingual dentin, d. P.d.m. = periodontal membrane. $\times 170$.
- FIG. 17. A midsagittal section of the upper incisor of a rat which was on the vitamin A-deficient diet for 49 days after weaning, illustrating the histology of the vesicle seen in the roentgenograms (Figs. 3 and 4). En.ep. = enamel epithelium; En.sp. = enamel space; D. = interglobular enamel-covered dentin; Od. = odontoblasts; Ost. = osteodentin within vesicle and adjacent to enamel space. $\times 85$.



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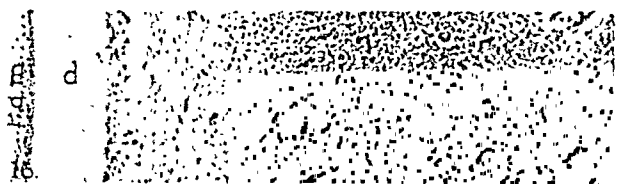
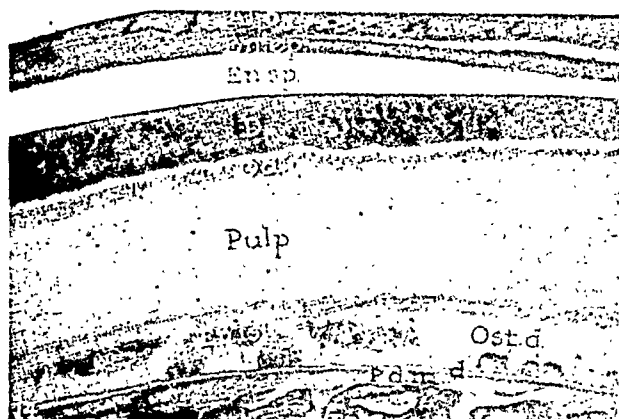


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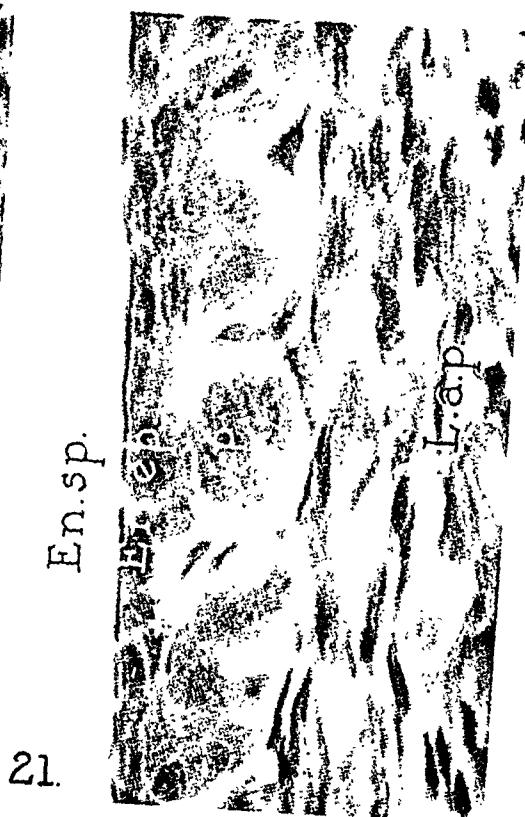
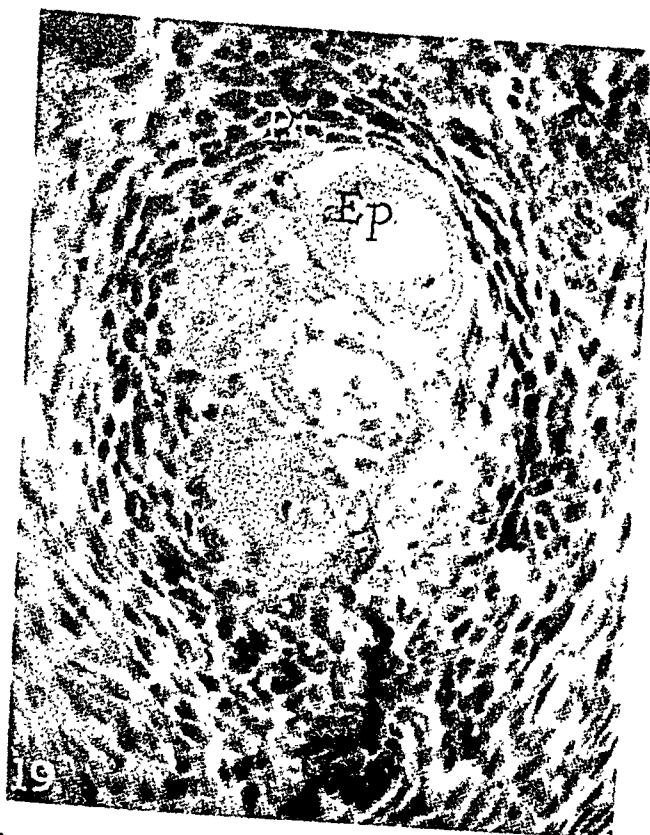
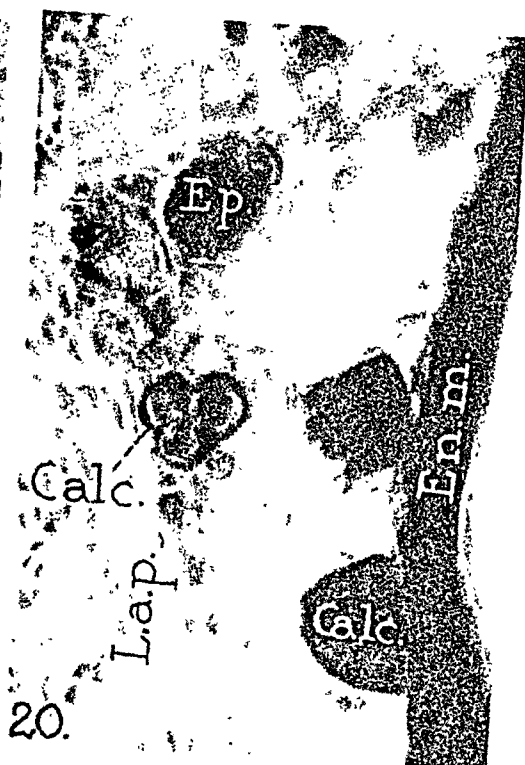
Incisor Teeth in Vitamin A Deficiency

PLATE 103

- FIG. 18. A high power field of a section of the distal portion of the pulp of the upper incisor of the same albino rat as seen in Figure 13. A layer of atypical dentin, *d*, has been deposited by the pulpal cells, under stimulation from the epithelial cells, *Ep.*, which arose from the odontogenic epithelium and proliferated in the pulp. *M.* = mesenchymal cells which have become only partially differentiated into odontoblasts. $\times 420$.
- FIG. 19. A high power field of the distal portion of the pulp of the upper incisor of a rat which had a similar history as that of Figure 18, showing the dense pulpal tissue, *P.t.* Some of the epithelial cells, *Ep.*, have degenerated and resemble a Hassall's corpuscle. $\times 420$.
- FIG. 20. Enamel hypoplasia in a field from the proximal area of the upper incisor of a rat which was on vitamin A-deficient ration for 38 days after weaning. Note degenerating epithelium, *Ep.*, which is giving rise to calcified masses or calcospherites, *Calc.* *En.m.* = organic enamel matrix; *L.a.p.* = connective tissue in labial alveolar periosteum. $\times 420$.
- FIG. 21. The distal third of the enamel organ of the upper incisor of a rat which was on vitamin A-deficient ration for 81 days after weaning, illustrating severe atrophic changes in the enamel epithelium, *En.ep.* The enamel papillae, *P.*, show at this level greater atrophy than is normally present. *En.sp.* = space formerly occupied by enamel which was lost in the decalcification process; *L.a.p.* = connective tissue of the labial alveolar periosteum. $\times 700$.

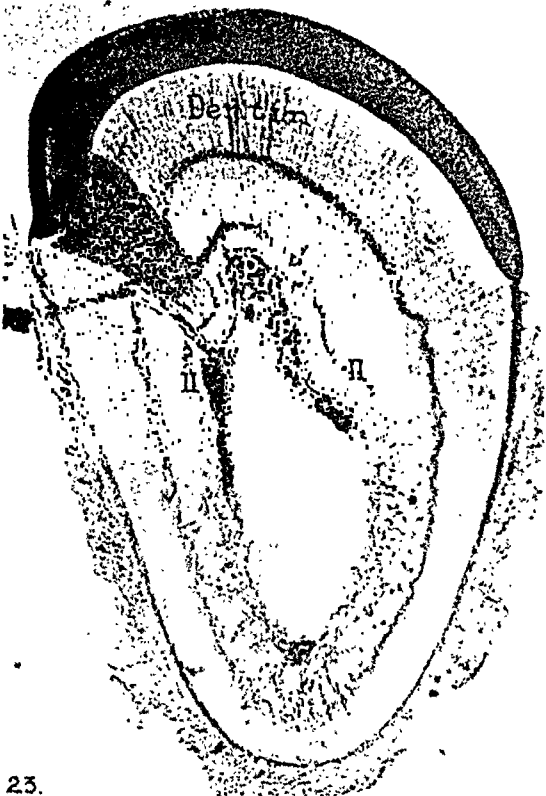
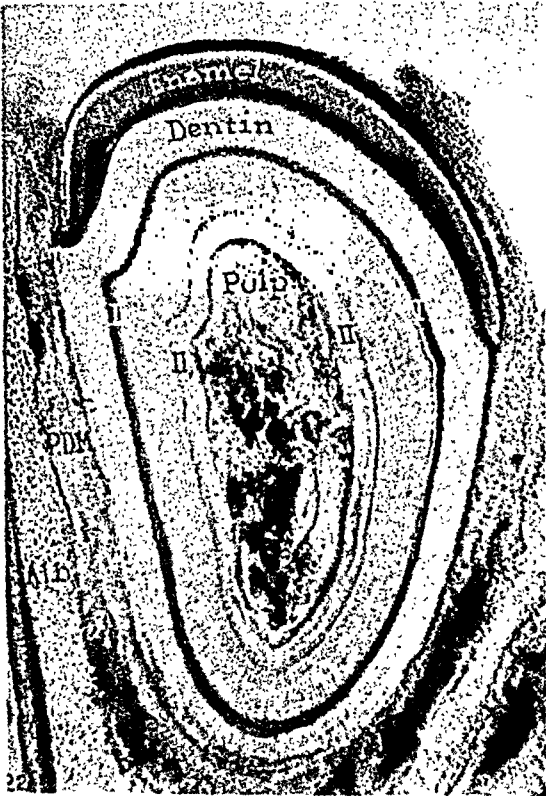


- FIG. 22. A transverse ground section of the lower incisor taken at the level of the mental foramen in a normal albino rat, 65 days old, which was given injections of alizarin red S on the 50th (I) and 60th (II) days. Note injection effects (I and II) and the uniform distance between them. Al.b. = alveolar bone; P.D.M. = periodontal membrane. See Text-Figure 2, A. $\times 90$.
- FIG. 23. A transverse ground section of the lower incisor taken at the level of the mental foramen in an albino rat, 65 days old, which was on a vitamin A-deficient diet since the 21st day of age. Injections of alizarin red S were given on the 50th (I) and 60th (II) days. Note injection effects (I and II) and the unequal distance between them. See Text-Figure 2, B. $\times 90$.
- FIG. 24. A midsagittal decalcified section of the upper incisor of an albino rat, 60 days old, showing the linguo-pulpal area in the proximal region. This rat was on vitamin A deficiency from the 21st to the 50th day and placed on vitamin A therapy from the 50th to the 60th day of age. Note the center core of epithelium, Ep., which arose during deficiency as a pulpal invagination of the lingual odontogenic epithelium, and the dentin, which was deposited as a result of the differentiation of the approximating mesenchymal cells (odontoblasts), Od., following the institution of vitamin A therapy. Replacement effects were noted as early as the third day after the beginning of therapy. Contrast with Figure 25. $\times 280$.
- FIG. 25. A midsagittal decalcified section of the upper incisor of an albino rat, 60 days old, showing a field similar to that in Figure 24. This rat was on a vitamin A-deficient diet since its 21st day. Note the epithelial cord, Ep., which arose as a pulpal invagination of the lingual odontogenic epithelium, and the absence of mesenchymal differentiation and dentin apposition. $\times 280$.
- FIG. 26. The lingual middle third of a midsagittal section of the upper incisor of an albino rat, 65 days old, which was on a vitamin A deficient diet since its 21st day. Note the abnormal fibrotic texture of the pulp which adjoins the amorphous lingual dentin. C. = cementum; P.D.M. = periodontal membrane. $\times 178$.
- FIG. 27. The apex of the distal root of the lower first molar of an albino rat, 65 days old, which was on a vitamin A-deficient diet since its 21st day. Note the abnormally prominent epithelial pearl, Ep., which is being engulfed by the amorphous secondary cementum, C. P.D.M. = periodontal membrane; Al.b. = fundic alveolar bone. $\times 480$.

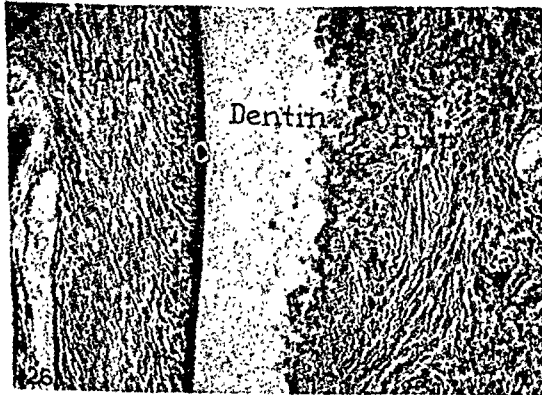
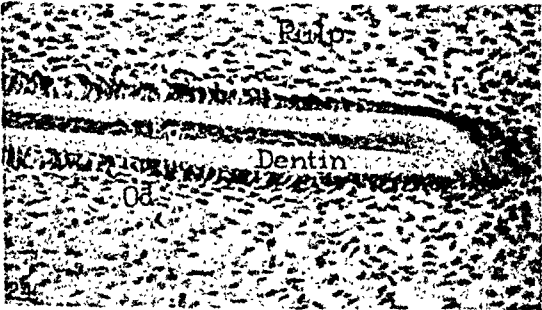


Schour, Hoffman and Smith

Incisor Teeth in Vitamin A Deficiency



23.



REPORTS OF CASES

Case 1

A colored girl, 16 years old, entered the hospital 3 weeks after giving birth to a full-term infant. The only anamnestic data available were that she had had convulsions. The patient died a few minutes after admission, so that clinical data are not available.

Gross Notes. Autopsy was performed 45 minutes after death. The most important findings were edema of the lungs, possible slight enlargement of the heart (270 gm.) and lack of significant changes in the internal genitalia. The kidneys weighed 340 gm. The capsules stripped very easily and the surface of the kidneys was pale, smooth and showed very few small hemorrhages. On section the cortex and medulla were well delimited, the medulla being a little darker than the cortex, and the glomeruli were enlarged, pale and plainly visible. The renal pelves showed nothing abnormal.

Microscopical Examination. The glomeruli were enlarged and very cellular. This cellularity was partly due to the presence of polymorphonuclear leukocytes, amongst which several eosinophilic leukocytes were found. The epithelium of the visceral layer of the capsule of Bowman was visible only on the outer surface of the glomerular loops and showed only a slight swelling and sometimes a few hyaline droplets. The outer basal membranes were clearly visible. The inner basal membranes were fused in many places; in others they were lying in close contact but were still visible as two separate membranes. In some places individual capillaries, sometimes containing a few erythrocytes, could still be distinguished. Many capillaries were fused and between the endothelial cells a surprisingly great number of very delicate, often branching, fibers were found. The fibers were much more delicate than those depicted by Bell⁵ and could not be called hyaline. Real hyalinization was absent. It was interesting to note that in ordinary paraffin sections of the same material, stained in the same way, the number of the individual fibers seemed much smaller than in the material first embedded in celloidin, whereas in the paraffin sections the closely adjacent basal membranes were distinguished with great difficulty. In the much reduced capsular spaces no red blood cells or fibrin were found.

THE OCCURRENCE OF MITOTIC DIVISIONS IN GLOMERULI IN GLOMERULONEPHRITIS AND MALIGNANT SCLEROSIS*

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The purpose of this publication is to prove that, contrary to current opinion, mitotic divisions in glomeruli in glomerulonephritis and malignant sclerosis are not exceptional. Though microscopic examination of the kidneys could be performed in but a small percentage of our material, yet in 3 of 30 cases thus examined from a series of 140 autopsies, mitotic divisions in glomeruli could be readily demonstrated in epithelial and endothelial cells.

MATERIAL AND METHODS

In cases Nos. 1 and 3, autopsy was performed 45 minutes after death, and in case No. 2, 30 minutes after death. Slices of renal tissue, not more than 3 mm. thick, were cut for fixation. In case No. 1, Stieve's solution¹ (saturated solution of corrosive sublimate, 76 cc.; undiluted commercial formaldehyde solution, 20 cc.; glacial acetic acid, 4 cc.) was used as fixative; in cases Nos. 2 and 3, a mixture of equal parts of a saturated solution of corrosive sublimate and of Bouin's solution. This mixture, recommended by Petersen² for embryological material, gives excellent results with surgical and autopsy material and is, in our opinion, much superior to Bouin's fluid alone. The material from cases Nos. 2 and 3 was embedded in paraffin after treatment with Peterfi's methylbenzoate-celloidin mixture.³ In case No. 1 it was deemed necessary to avoid as much as possible the shrinkage often apparent in paraffin sections. Therefore, the tissues were dehydrated in glycerin,⁴ soaked for 3 days in a mixture of five parts of a 6 per cent celloidin solution and one part of oil of cedarwood; hardened in anhydrous chloroform and thereafter embedded in paraffin after passing through benzene and benzene-paraffin. Sections were cut 5 and 6 μ thick and stained with azocarmine-aniline blue and hematoxylin-azophloxin.

* Received for publication December 21, 1940.

Microscopical Examination. The glomeruli were not enlarged. The basal membranes in nearly all glomeruli showed thickening, which was in general not very pronounced but was more strongly developed in individual glomeruli or in some loops of a glomerulus. Sometimes it appeared that the basement membrane, especially in the peripheral parts of the loops, was split longitudinally and surrounded the endothelial cells. Also, that small short fibers split off from the basement membrane. The endothelial nuclei were distinctly increased. The epithelial cells of the visceral layer of the capsule of Bowman were very conspicuous. Their protoplasm and nuclei were swollen and they often contained vacuoles and hyaline droplets. In several glomeruli, mitotic divisions were found and it could easily be ascertained that the dividing cells were lying outside of the basement membrane and belonged to the visceral layer of the capsule. The tubules were often widened and contained granular and hyaline casts. Many epithelial cells were swollen and showed hyaline droplet degeneration; in other tubules the epithelial cells were flattened. In the epithelium of the tubules many mitotic divisions were found. The interstitium was edematous and contained very few lymphocytes and some histiocytes.

Epicrisis. On purely morphological grounds we believe this case to be an instance of glomerulonephritis closely related to lipid nephrosis (Bell⁶). Mitotic divisions were found in the epithelial cells of the glomeruli.

Case 3

A colored man, 37 years old, entered the hospital complaining of dullness, headache which had been increasing for several days, and vomiting of blood. On admission the heart was enlarged and the pulse rate was 125 per minute. Examination of the ocular fundi was impossible. The blood pressure was 170 systolic and 120 diastolic. Examination of the urine showed the specific gravity to be 1006; albumin and glucose, negative; urobilin, positive; acetone, negative; red blood cells, none; casts, none. The blood showed the hemoglobin to be 65 per cent; red blood cells, 3,900,000; sedimentation rate, 54 to 86 mm. (Westergren); Wassermann's and Kahn's tests, negative; nonprotein nitrogen, 300 mg. per cent, and blood urea, 280 mg. per cent. The electrocardiogram showed a serious myocardial lesion, probably a bundle-branch block. Tests of kidney function could not be performed.

On the day of admission the patient vomited a few black coagula and some fresh blood. Thereafter the vomiting of blood stopped, but the condition of the patient deteriorated rapidly. On the fourth day Cheyne-Stokes' breathing appeared and the patient died 7 days after admission.

In many glomeruli, mitotic divisions were found, sometimes three in one glomerulus. The distribution of the mitoses over the different glomeruli was unequal. In some sections many glomeruli had to be examined before a mitosis was found; in other sections they were detected quickly and easily. The mitotic divisions were found in all stages, ranging from the spirem to the diaster. With the azocarmine stain the centrioles and achromatic spindles were easily demonstrated, so that it was impossible to mistake dark-staining, degenerating nuclei of leukocytes for mitotic figures. In some cases, as illustrated in Figures 1 and 2, the dividing cell could, by its location, be recognized as an endothelial cell, as it was lying just inside the outer basement membrane. In the epithelial cells mitoses could not be found. The tubules contained little granular material and the epithelial cells were swollen and sometimes showed hyaline droplets. Only in very few tubules were red blood cells found. The interstitial tissue was unchanged.

Epicrisis. This is a typical example of acute glomerulonephritis showing intracapillary fibers, polymorphonuclear leukocytes and proliferation of the endothelial cells. In the glomeruli typical mitoses could be demonstrated and in several instances the dividing cell could be recognized as endothelial.

Case 2

A colored man, 40 years old, entered the hospital and died 1 hour after admission. Clinical and anamnestic data were not available.

Gross Notes. Autopsy was performed 30 minutes after death. The most important findings were as follows: The kidneys were enlarged, the left kidney weighing 365 gm. and the right 315 gm. Their consistence was diminished, the capsule stripped easily and the surface was smooth and yellowish gray. On section, the cortex was widened, welled up above the cut surface, and its color was gray-yellow, with deeper yellow streaks. The liver was enlarged, weighing 2300 gm., and was yellowish light brown in color, with small yellow spots. The spleen was enlarged, weighing 285 gm., and showed on section many yellowish white spots on a red background. The heart was not enlarged and weighed 245 gm. The heart muscle was brown-red. Retroperitoneal lymph nodes were swollen, moist, and whitish. There was only slight edema.

DISCUSSION

As Bell⁵ stated, it is generally agreed that the essential lesion in glomerulitis is an increase in the number and size of the endothelial cells. On the other hand, most authors agree on the absence of mitotic divisions in the endothelial cells; neither are mitoses in the epithelial cells mentioned in recent publications. Bell therefore concluded that if cell division actually occurs, it is largely of the amitotic type. As experienced cytologists either consider amitosis very rare in mammals (Levi⁷) or doubt the existence of real amitosis (Maximow and Bloom⁸), this is not a satisfactory solution of the problem. Other authors state simply that there is an increase in the number of the endothelial cells (Kimmelstiel and Wilson⁹) without telling how this increase is brought about, and this is true also of many textbooks (Aschoff,¹⁰ Hueck,¹¹ Fishberg,¹² Hadfield and Garrod¹³). Van Waveren¹⁴ assumed that the endothelial nuclei always outnumber epithelial, though Bell⁵ and especially von Möllendorff,¹⁵ the latter using the most excellent histological technic, came to directly opposite conclusions. As one of us worked in the same laboratory as van Waveren and performed part of the autopsies from which he obtained his material, we are in a position to confirm Bell's⁵ opinion that the material and methods of van Waveren were quite unsuited for these investigations. Only Kaufmann¹⁶ records the finding of mitoses in "adventitial cells" of the capillaries of the glomeruli.

It is a well known fact that after death the number of mitoses found in a given tissue decreases with time (Schmorl,¹⁷ Mallory¹⁸) and it is therefore not surprising that in tissues, fixed many hours after death, the number of mitoses found may be small, even in rapidly growing tissues. Furthermore many mitoses become indistinct (Casey¹⁹) and it is difficult to distinguish them from the nuclei of degenerating cells. In connection with this it is interesting to note a quotation from Karsner, Saphir and Todd,²⁰ by MacMahon,²¹ who was the first to describe the regeneration of heart-muscle fibers in infants. Failing to find mitotic figures in hearts of adults these authors remarked that this was perhaps due to the fact that the hearts were obtained *post mortem*.

In our cases the autopsies were performed a very short time after death, thin slices of tissue were fixed and rapidly penetrat-

Gross Notes. Autopsy was performed 45 minutes after death. The principal findings were as follows: The heart was enlarged, weighing 545 gm. There was extensive necrosis in the wall of the left ventricle and in the papillary muscles, less extensive in the wall of the right ventricle, and coronary sclerosis, especially of the ramus descendens anterior sinister, of which the lumen was very much reduced. Typical syphilitic lesions were present in the aorta and in the innominate and subclavian arteries. The kidneys were enlarged, the right and left kidneys weighing respectively, 255 and 215 gm.; their consistence was diminished, the capsules stripped easily, the surface was nearly smooth and the color was grayish brown with the admixture of some yellow, showing, in addition, very numerous irregular red spots of the size of a pinhead, sometimes even a little larger. On section the cortex welled up, the demarcation between cortex and medulla was not distinct and many small irregular red spots were visible. The pelvis showed nothing of significance. The blood vessels on section did not project and their lumina were open. No gross lesion, which could have caused the vomiting of blood, was found.

Microscopical Examination. Most arterioles showed an extensive hyalinization and fatty degeneration. In many places arteriolonecrosis or endarteritis was observed. The glomeruli showed a variety of lesions; glomerulonecrosis, hemorrhages, necrosis of some capillary loops and in many instances alterative or proliferative glomerulitis. Others showed a progressive hyalinization, although still others appeared quite normal. In the glomeruli showing alterative or proliferative glomerulitis, the epithelial cells were swollen and sometimes giant cells with three or more small nuclei were found. Hyaline droplet degeneration was quite common. In the glomeruli with proliferative glomerulitis the endothelial nuclei were increased. Mitotic divisions were found in epithelial and endothelial cells. Many tubules were filled with erythrocytes or with hyaline or granular casts. Hyaline droplet degeneration was quite frequent. Small areas with atrophic tubules and infiltrates of lymphocytes were found.

Epicrisis. We believe this to be a case of malignant sclerosis, complicated by luetic aortitis and infarction of the heart. That the blood pressure was not so high as usual was probably due to the cardiac failure. In the glomeruli, mitoses were found in both endothelial and epithelial cells.

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DESCRIPTION OF PLATES

PLATE 105

- FIG. 1. Case 1. A glomerulus with a mitosis in its upper left corner and in the center of the field. $\times 716$.
- FIG. 2. Case 1. The same mitosis at a higher magnification. The dividing cell is lying inside the basement membrane and must be considered as endothelial. One centrosome is visible. $\times 1500$.
- FIG. 3. Case 2. A mitosis in an epithelial cell. Centrosomes and spindles are plainly visible. $\times 1500$.

ing fixatives were used. We believe that these factors enabled us to find the mitotic divisions. When we compare sections of other organs from our autopsy material, 50 per cent of which is fixed less than 1 hour after death, with those from other laboratories in which we have worked and where the interval between death and autopsy averaged more than 24 hours, the difference in the number of mitotic divisions is striking. The same is true of surgical material instantly cut into thin slices and fixed after removal from the body as compared with large specimens, often whole organs or large tumors, when left untouched for some time or placed in their entirety into a fixative. Such material is, of course, quite sufficient for diagnostic purposes but is not suitable for delicate histological work, a fact often forgotten by pathologists.

SUMMARY

A brief description of one case of acute glomerulonephritis, one case of subacute glomerulonephritis and one case of malignant sclerosis is given. In the first case mitotic divisions were found in the endothelial cells of the glomeruli, in the second case in the epithelial cells of the glomeruli, in the third case in both endothelial and epithelial cells.*

The importance of early and good fixation for the study of the glomerulus, and especially for the finding of mitotic divisions, is stressed.

* After submitting this paper we examined the kidneys of a man dying from septicemia with widespread metastatic abscesses. A few mitotic divisions were found in the endothelial cells of the glomeruli. The autopsy was performed 5 minutes after death.

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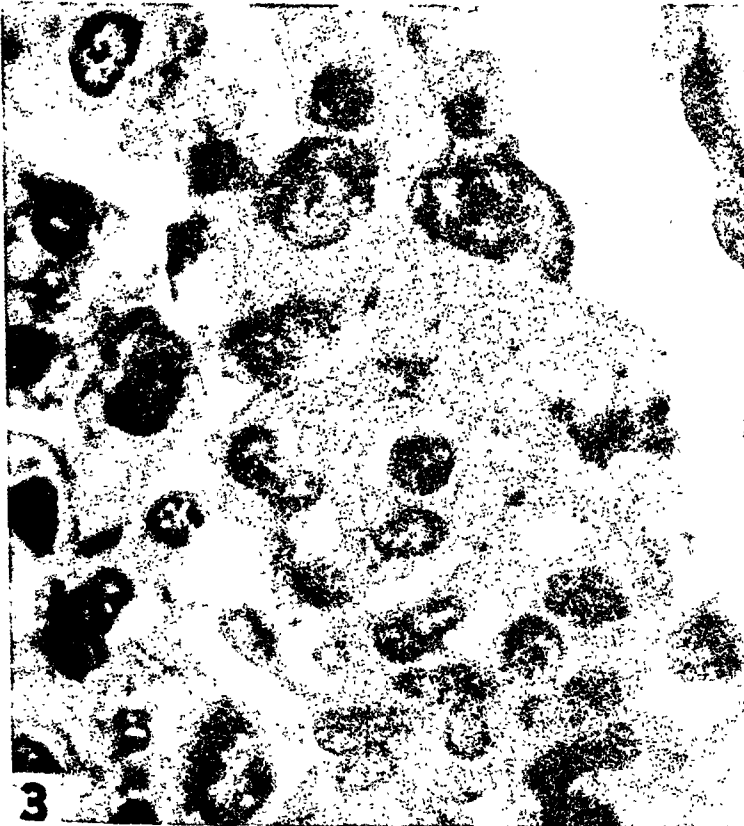
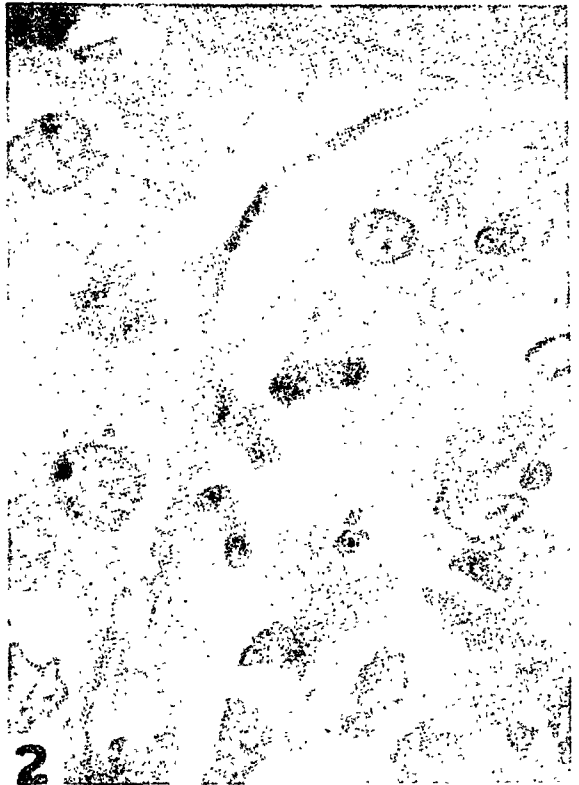
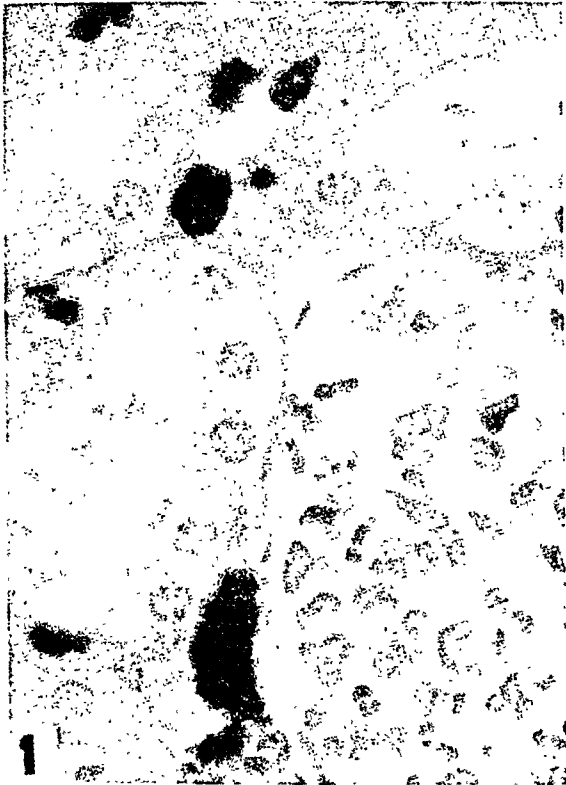
PLATE 106

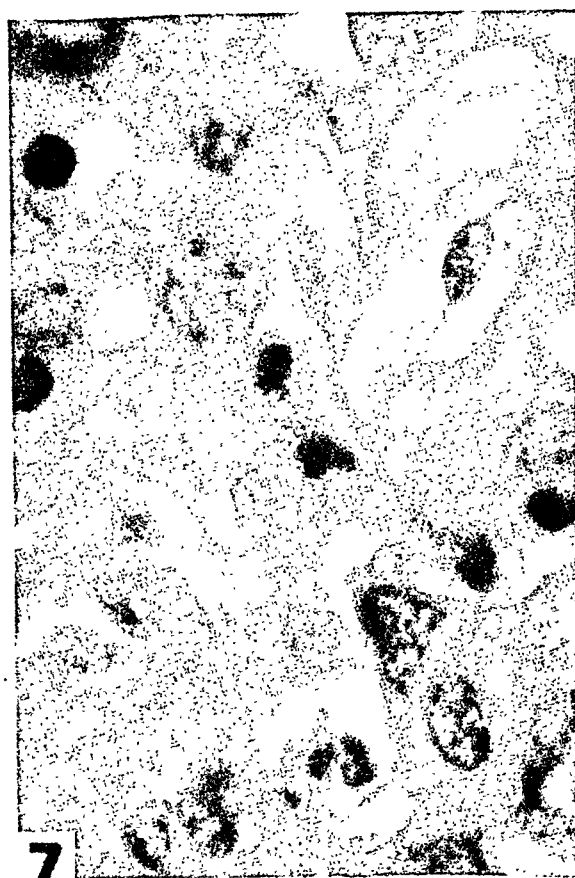
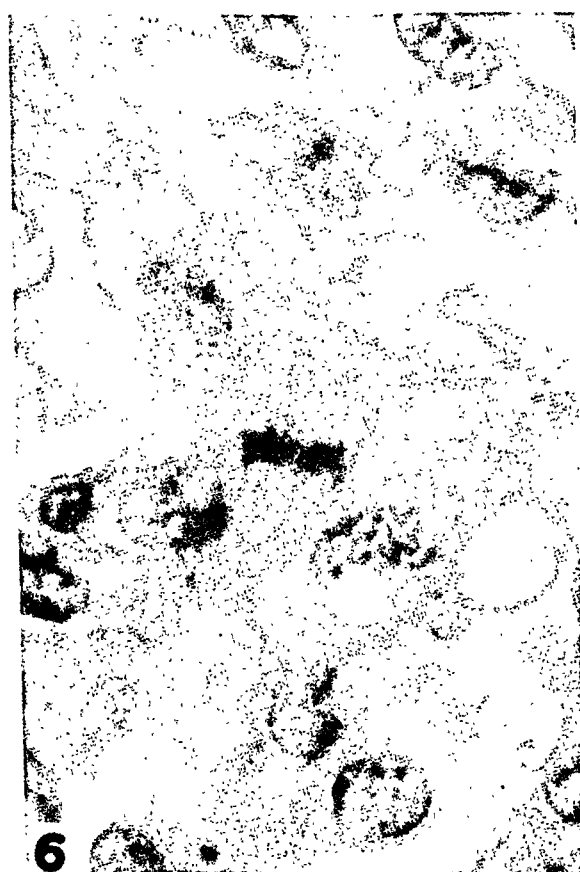
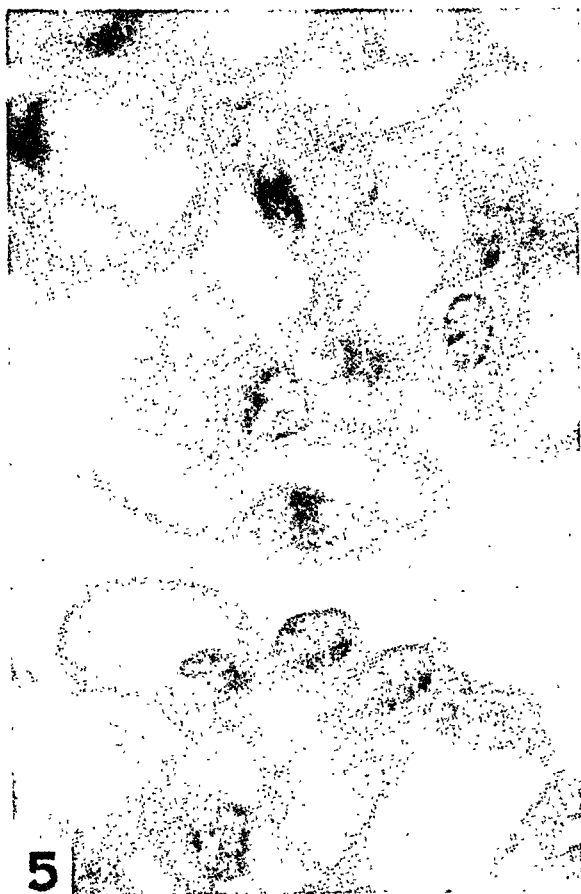
FIG. 4. Case 2. A mitosis in an epithelial cell. $\times 1500$.

FIG. 5. Case 3. A mitosis in an endothelial cell. $\times 1500$.

FIG. 6. Case 3. Two mitoses in epithelial cells at the vascular pole of a glomerulus. $\times 1500$.

FIG. 7. Case 3. A diaster in a glomerulus. $\times 1500$.





FORTY-FIRST ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

NEW YORK CITY
APRIL 10TH AND 11TH, 1941

The Secretary announced the election of Tracy B. Mallory as Assistant Editor of the *American Journal of Pathology*, and J. Harold Austin to membership on the Editorial Board for a term of six years beginning January 1, 1942, to succeed H. Gideon Wells whose term expires December 31, 1941.

The Secretary announced that the Council had voted to issue a special number of the *American Journal of Pathology* in honor of H. Gideon Wells.

The Council recommends that in Article 2 of the By-Laws the sentence "The Council shall consist of seven Members elected by the Association, and the Secretary and Treasurer ex officio" be changed to read "The Council shall consist of seven Members elected by the Association, and the Secretary, Treasurer and Editor of the *American Journal of Pathology* ex officio."

Voted to accept with regret the resignations of R. L. Cecil, R. S. Cunningham, H. A. Kemp, H. McL. Kinghorn, F. A. McJunkin, Carl TenBroeck and R. L. Thompson.

Voted to record with deep regret the deaths of Maude E. Abbott, D. M. Cowie, O. I. Cutler, J. G. Fitzgerald, J. A. Lanford, David Perla, Ernest Pribram, D. G. Richey, Kurt Semsroth and Hans Zinsser.

The Secretary announced that the next meeting of the Association will be held as the guests of Washington University in St. Louis, Missouri, April 2 and 3, 1942.

The Secretary announced that it had been voted to have a symposium on "Neoplasms of Endocrine Glands" and to appoint Howard T. Karsner as referee.

The Secretary announced the election of N. Chandler Foot as delegate of the Association to the Executive Committee of the Congress of American Physicians and Surgeons and Irving Graef as alternate.

The Secretary announced the election of Samuel R. Haythorn as delegate of the Association to the Celebration of the Fiftieth Anniversary of the University of Chicago and of Louise Pearce as delegate to the Celebration of the One Hundred and Seventy-Fifth Anniversary of Rutgers University.

THE AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

Forty-First Annual Meeting, New York University Medical
College, New York City

April 10th and 11th, 1941

PRESIDENT BAYNE-JONES IN THE CHAIR

BUSINESS MEETING

April 10th, 1941

On nomination of the Council the following officers were
elected by the Association:

<i>President</i>	SAMUEL R. HAYTHORN
<i>Vice-President</i>	PAUL R. CANNON
<i>Secretary</i>	HOWARD T. KARSNER
<i>Treasurer</i>	ALAN R. MORITZ
<i>Incoming Member of Council</i>	ERNEST W. GOODPASTURE
<i>Assistant Secretary</i>	FRANCIS BAYLESS
<i>Assistant Treasurer</i>	GRANVILLE A. BENNETT

The Secretary announced the election of the following new
members:

Lauren V. Ackerman	Robert Hebbel
Joseph A. Beeman	Edgar S. Ingraham
Gerson R. Biskind	William Kaufmann
William C. Black	Donald H. Kaump
Mark M. Bracken	Simon Koletsky
Lloyd Catron	Joseph R. Kriz
A. Reynolds Crane	Herbert Lund
Malcolm B. Dockerty	John C. McCarter
Jesse E. Edwards	Anderson Nettleship
W. Norman Elton	Lincoln Oppen
Cyrus C. Erickson	Bjarne Pearson
J. Howard Ferguson	Donald J. Rehbock
James S. Forrester, Jr.	Seaton Sailer
Raymond Gettinger	Murray Sanders
Arthur M. Ginzler	George A. C. Snyder
Ralph M. Hartwell	Leon J. Tragerman

Jacob Werne

innumerable acid-fast bacilli. Animals which die or are sacrificed when their skin sensitivity is at the 0.01 mg. level show only proliferative tubercles in the lungs. In these tubercles acid-fast bacilli are demonstrated with difficulty.

THE STRUCTURE OF BACTERIA AS SHOWN BY THE ELECTRON MICROSCOPE.
Stuart Mudd and (by invitation) T. F. Anderson, K. Plevitsky and H. E. Morton, Philadelphia, Pa.

Abstract. Micrographs of bacteria made with the RCA electron microscope have shown very definite differentiation between a solid outer cell wall and an inner fluid, or potentially fluid, protoplasm. In chains of *Streptococcus pyogenes* and of aerobic spore-bearing bacilli the continuity of the chain has been shown to be due essentially to the continuity of the outer cell walls. Division in a strain of *Str. pyogenes* appears to be accomplished by pinching off the cell wall and its contained protoplasm. In species of the genus *Bacillus*, intercellular plates, as described by Knaysi and others, may be seen. Disruption of streptococci and bacilli by sonic vibration permits escape of the inner protoplasm, leaving the cell walls as "ghosts."

Cells of *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae* have been relatively transparent to the electron beam. In tubercle bacilli opaque granular bodies of various sizes may be seen in the inner protoplasm. In diphtheria bacilli grown on blood agar, very striking polar granules are found. In diphtheria bacilli grown on potassium tellurite medium, needle-shaped crystals of metallic tellurium are clearly visible within the cell protoplasm. Shaking such bacterial cells with bromine water dissolves the crystals, a behavior which is to be expected with tellurium metal.

Discussion

(Dr. Alex B. Ragins, Chicago, Ill.) I should like to ask Dr. Mudd whether bacteria of the Gram-positive series become Gram-negative when protoplasmic regression is demonstrated.

(Dr. Mudd) When the bacilli are fragmented in the sonic vibrator, the disintegrated or injured cells are Gram-negative. The uninjured cells remain Gram-positive.

(Dr. M. H. Soule, Ann Arbor, Mich.) How do you harmonize the opacity of *Bacillus megatherium* in this type of microscopy with the seeming transparency of this form under the dark field?

(Dr. Mudd) I have not time to go into the optics of this, but conditions are entirely different. Light goes through glass or quartz without interruption. Electrons are stopped by anything, even by air; all of this electron microscopy has to be done in a high vacuum. The lightness and darkness in an electron picture, as in an X-ray picture, are determined by the density and thickness of the object.

THE ELECTRON MICROGRAPHY OF PURIFIED VIRUSES. W. M. Stanley and T. F. Anderson (by invitation), Princeton and Camden, N. J.

Abstract. Viruses range in size from about 300 m μ to about 10 m μ . There has been considerable difficulty in their microscopy, since the limit of resolution for visual light is about 250 m μ . However, the recent development of an electron microscope with a resolving power extending down to about

SCIENTIFIC PROCEEDINGS

THE COMPLEMENT-FIXATION TEST IN THE DIAGNOSIS OF SOME TYPES OF HUMAN ENCEPHALITIS. J. Casals and R. Palacios (by invitation), New York, N. Y.

Abstract. Fresh sera from 13 patients with encephalitis, previously diagnosed on the basis of neutralization tests, have been examined for specific complement-fixing antibodies with the following results: Specific fixation was obtained with sera drawn from 2 persons 8 years after an attack of louping-ill; from 5, 2½ years after an attack of Eastern equine encephalomyelitis, and from 2, 2½ years after Western equine encephalomyelitis; from 2, 4 to 6 months following St. Louis encephalitis, and from 2, 2 to 3 weeks after lymphocytic choriomeningitis. No fixation was obtained with sera drawn from 4 patients 4 to 8 years after an attack of St. Louis encephalitis; from 1, 5 years after lymphocytic choriomeningitis; from 2, with rabies, nor from 5 with fresh, undiagnosed, clinical encephalitis.

Discussion

(Dr. Leslie T. Webster, New York, N. Y.) The practical implication of this work is the development of a method for clinical diagnosis of the several virus encephalitides. Hitherto, the clinician has depended upon the neutralization test, a somewhat laborious procedure which cannot readily be carried out in all hospital laboratories but only in those in which the various viruses are maintained and tests are being run routinely. Dr. Casals' complement-fixation test is simple and practical. It remains to be seen how soon after onset of disease complement-fixing antibodies appear, in what proportion of cases, and how long they persist.

(Dr. S. Bayne-Jones, New Haven, Conn.) Dr. Casals gave the incubation temperature for rabbit, mice and guinea pig sera. Did you speak of the human serum incubation temperature?

(Dr. Casals) No; the human sera are usually incubated at 60° C. except in two instances. Wassermann-positive sera give a nonspecific reaction similar to the rabbit sera and must be incubated at 65° C. Sera from a few patients with encephalitis give a nonspecific reaction at 60° C. but on heating the serum at 65° C. the nonspecificity disappears.

THE CORRELATION BETWEEN ANATOMICAL CHANGES AND THE ALLERGIC STATE IN TUBERCULOUS GUINEA PIGS. C. E. Woodruff, H. S. Willis and (by invitation) Ruby G. Kelly, Northville, Mich.

Abstract. Guinea pigs were infected with 0.1 mg. of virulent tubercle bacilli. From the data obtained by testing these pigs at intervals of 2 weeks, with varying concentrations of O.T., skin sensitivity curves have been constructed. The healthiest animals reach a plateau of maximum skin sensitivity about 2 months after infection. At this time they give a definite reaction to 0.01 mg. O.T. Some animals fail to reach this plateau or, after reaching it, show a rapid decline in the level of skin sensitivity. The animals whose skin sensitivity falls below the 1 mg. reacting level at autopsy invariably show lungs filled with areas of tuberculous pneumonia in which may be demonstrated

to identify it because of its distinctive shape. I think it would be possible for a particle of bushy stunt virus to become occluded mechanically in a precipitate formed by tobacco mosaic virus and its antiserum, and we would not see it. In this case, there might be a small amount of mechanical occlusion, but certainly the great mass of material reacts specifically.

THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS WITH PROMIN (SODIUM SALT OF P,P' DIAMINO-DIPHENYL-SULFONE-N,N'-DEXTROSE SULFONATE). William H. Feldman and (by invitation) H. C. Hinshaw and H. E. Moses, Rochester, Minn.

Abstract. Continuing a study previously reported on the effect of promin on experimental tuberculosis, 80 guinea pigs were inoculated with human tubercle bacilli (strain H37RV). Sixty-eight animals were treated with promin and 12 were kept as controls. Chemotherapy was started before infection in 20 animals, and in 48 animals treatment was withheld for varying periods up to 6 weeks after infection with tubercle bacilli. The experiment was terminated after 191 days. Results were as follows: Extensive and progressive tuberculosis in the controls and slight or no gross signs of tuberculosis in the treated animals. In only 3 of the treated animals was tuberculosis recognized grossly in the spleen. In the others that were treated with promin, visceral tuberculosis was apparently absent. The results suggest that under the conditions of the experiment promin proved to be an agent of considerable effectiveness in successfully combating experimental tuberculosis in guinea pigs.

Discussion

(Dr. Charles E. Woodruff, Northville, Mich.) I am very much interested in this paper. We attempted to treat two groups of guinea pigs, of 10 each, infected with tubercle bacilli, with promin given subcutaneously and noticed no beneficial effects. As a matter of fact, the mortality in pigs which received daily subcutaneous injections of promin was rather high. It is of considerable interest that in these animals which were fed promin the drug apparently had a beneficial effect.

(Dr. Feldman) We have had no experience with the drug given subcutaneously.

(Dr. M. M. Steinbach, New York, N. Y., by invitation) I would like to ask why the drug was given by mouth, as there are some indications that it is toxic by that method. Is that true for man or guinea pig?

(Dr. Feldman) We gave the drug by mouth because we were too lazy to give it four times a day parenterally.

(Dr. Steinbach) I think Dr. Sharpe, Medical Director of Parke, Davis and Co., indicated in a private communication that it was rendered toxic when given by mouth.

(Dr. Feldman) This compound is not pleasant to take when you attempt to give it in undiluted form. The taste simulates a mixture of onion and asafetida. When mixed with the feed we added karo syrup which veiled the taste effectively, and the animals not only ate it well, but many of them doubled their weight.

(Dr. Steinbach) We are using it in tuberculous guinea pigs, giving it subcutaneously three times a day, and I admit it is a nuisance to inject the

5 $m\mu$ has made it possible to make micrographs of these small infectious agents. Micrographs of several purified viruses and of mixtures of viruses with homologous and heterologous antisera were shown.

Discussion

(Dr. Paul R. Cannon, Chicago, Ill.) I should like to ask Dr. Stanley if he were able to tell whether the antibody was dispersed evenly around the virus molecule or whether it was attached irregularly. The purpose of my question is to learn whether this method offers any additional information as to whether the antigen-antibody reaction is a physical adsorption or a chemical union to polar groups.

(Dr. Stanley) We can get some information, because the diameter of the molecule of the tobacco mosaic virus is increased from its normal value of about 15 $m\mu$ to a value of about 60 $m\mu$ following reaction with its antiserum. Since the molecule is a long rod about 280 $m\mu$ in length, it would appear that the entire molecule is covered and that the reaction is not localized at one single spot on the virus molecule. Since the size of the usual rabbit antibody molecule is about 3.7 $m\mu$ in diameter and about 27 $m\mu$ in length, it is obvious that the ends rather than the sides of the antibody molecules must become attached to the virus molecules in order to account for the observed increase in the diameter of the virus molecule.

You asked whether this is a physical adsorption or a chemical union. The fact that we observe no reaction between anti-bushy stunt virus serum and tobacco mosaic virus, or between anti-tobacco mosaic virus serum and bushy stunt virus indicates a remarkable degree of specificity. I think that this specificity must be similar to that which obtains in any ordinary chemical reaction.

(Dr. Stuart Mudd, Philadelphia, Pa.) There is one observation I should like to make regarding the remarkable agreement between the conclusions that have been derived from indirect measurements on the size and shape of virus particles by the various physical-chemical methods used, the conclusions which the immuno-chemists have derived from the reactions of antigen and antibody, and the results obtained by electron microscopy. The electron pictures confirm the conclusions from indirect methods to a remarkable degree.

(Dr. Thomas Francis, Jr., New York, N. Y.) May I ask whether the evidence is sufficiently clear so that you can say that the agglutination is entirely specific and that there is no foreign particle in these aggregations?

(Dr. Stanley) As you could see from the slides, the molecules of tobacco mosaic virus are rods about 15 $m\mu$ by 280 $m\mu$, whereas the particles of bushy stunt virus are spheres about 26 $m\mu$ in diameter. The distinctive shapes and sizes of these two viruses, plus the fact that their rabbit antisera were available, provided the incentive for these studies. Although we are not immuno-chemists, it was obvious that these materials afforded a unique opportunity to study the antigen-antibody reaction under the electron microscope. The micrographs show that in a mixture of the two viruses with anti-bushy stunt virus serum, the tobacco mosaic virus molecules are unaffected and do not appear to be occluded in the precipitate formed as a result of the reaction between bushy stunt virus and its antiserum. The proof is rather good, for if tobacco mosaic virus were present in this precipitate it would be possible

films in areas where this disease may be expected. I should like to ask two questions: first, does Dr. Butt think there might be other mycotic lesions which have a similar calcification? I ask that because there is one very large area in this country, the region in the east-central part of the United States, in which calcific lesions are very common in people who do not react to tuberculin. A certain percentage of these people, but not all, appear to react to coccidioidin; as far as I know there has been no direct association established between reactions in that area and such calcific lesions as you have shown. The second question I want to ask was touched upon, and that is whether a real primary complex developed?

(Dr. Butt) In answer to your second question concerning the relationship of the primary coccidioidomycotic lesion to the primary complex of tuberculosis, it is my impression that they are identical. The primary lesion of coccidioidomycosis is in the periphery of the lung with extension to the peribronchial lymph nodes. The first case presented today is an excellent example. A healed lesion was found in the upper lobe of the right lung, indistinguishable grossly from a primary tuberculous infection. Also healed lesions and the spherules of *C. immitis* were found in the peribronchial lymph nodes.

We have considered the possibility of other mycotic infections as the cause of calcified lesions in the lungs and peribronchial lymph nodes. So far, however, we have not demonstrated other causes for such healed or arrested pulmonary lesions. When one considers that in the past few years we have had almost as many autopsied cases of torulosis as we have had of the disseminated form of coccidioidal granuloma, the question arises whether or not some of these healed lesions are the result of a torula infection.

(Dr. William H. Feldman, Rochester, Minn.) Have you made studies of the histopathology of the local skin reaction in the positive cases?

(Dr. Butt) These are living patients, and usually the lesion has disappeared by the time of autopsy.

(Dr. Feldman) I had in mind material obtained by biopsy.

(Dr. Butt) No, we have not examined biopsy specimens.

SOME PATHOLOGICAL ASPECTS OF HUMAN MALARIA. Paul R. Cannon, Chicago, Ill.

Abstract. Material was presented contrasting the pathologic effects in 2 patients who died during active malarial infection. In 1 patient the infection was of a benign tertian type, whereas in the other it was malignant estivo-autumnal. Both patients were in middle age. Both were untreated so far as malaria was concerned and the duration of active symptoms was approximately the same, being 9 days in the case of the malignant malaria, and 11 in the benign infection. Histopathologic examination revealed the following:

In the benign infection malarial parasites had practically disappeared from the blood stream and only residual pigment could be seen in the spleen, liver and, to a lesser degree, in the bone marrow. No evidences of phagocytosis could be seen in any other organ examined, indicating that in the early stages of the benign infection the parasites were eliminated practically exclusively by these three organs. The lymphoid structures of the spleen were entirely normal, and there was no evidence of toxic effect upon the hepatic cells. In the patient dying from malignant malaria, on the other hand, the parasites were particularly numerous in certain regions. There was an enor-

animals every 8 hours. In some animals we are observing hemorrhages at the site of injection.

(Dr. Raymond H. Goodale, Worcester, Mass.) May I ask the doses given the guinea pigs?

(Dr. Feldman) We kept the dose at 300 mg. per animal for each 24 hours

(Dr. Goodale) What was the total dosage given the animals?

(Dr. Feldman) My mathematics is not sufficiently good for an immediate answer.

A STUDY OF LATENT LESIONS OF COCCIDIOIDOMYCOSIS CORRELATED WITH COCCIDIOIDIN SKIN TESTS. E. M. Butt and (by invitation) Arthur Hoffman, Los Angeles, Calif.

Abstract. Among 431 adult males on the medical service of the Santa Fe Coast Lines Hospital, a positive coccidioidin skin test was obtained in 18.7 per cent. These patients were from various cities and towns of California, Arizona and New Mexico. Fifty per cent of the patients from the San Joaquin Valley reacted positively to the coccidioidin skin test, whereas for the San Francisco area the figure was 23 per cent. The percentage of positive reactors from Arizona and New Mexico was 11.9 and 10.5 per cent respectively.

The lungs of patients coming to autopsy were removed intact and examined by X-ray for fibrotic and calcified lesions. Such lesions were excised, cultured and in some instances injected into guinea pigs. Eleven of the 431 cases have been autopsied. Four of the 11 had positive coccidioidin skin tests. No lesions suggestive of an arrested or healed coccidioidal granuloma were found in the negative reactors. Five of the 7 negative cases had evidences of a healed tuberculosis. Animal inoculations and cultures of this material were negative.

A positive culture of *Coccidioides immitis* was obtained from 1 of the 4 positive cases. Histologically the spherules of the fungus coccidioides were found in 3 of the 4 positive reactors.

The pulmonary lesions consisted of small encapsulated areas of caseation located in the parenchyma of the lungs. Histologically the capsules were noted to be composed of dense hyalinized fibrous tissue in which there were varying amounts of calcium. The centers were caseous. In all 4 cases arrested or healed lesions were found in the peribronchial lymph nodes. Spherules were demonstrated in the peribronchial lesions in 2 of the 4 cases.

Discussion

(Dr. Max Pinner, Bedford Hills, N. Y.) I should like to ask whether tuberculin tests were made in any of the positive cases.

(Dr. Butt) We have started another series of 500 cases that are being skin-tested with coccidioidin. Tuberculin skin tests are being performed on the positive reactors to coccidioidin. So far there has been no correlation between the positive reactors to coccidioidin and the tuberculin skin tests. About one half of the cases reacting positively to coccidioidin react positively to the tuberculin test. In the series of 431 cases reported, no tuberculin skin tests were performed.

(Dr. Esmond R. Long, Philadelphia, Pa.) This is a very important finding because it makes it necessary for us to revise our interpretation of X-ray

(Dr. Jacob Furth, New York, N. Y.) In regard to the specificity of this phenomenon described by Dr. Cannon it is noteworthy that a similar phenomenon occurs in leukemia, namely, leukostasis. The accumulation of primitive blood cells in the capillaries is likewise "selective" in leukemia, and there are often, side by side, capillaries distended with leukemic cells and capillaries filled with normal blood. It is unlikely that antibody formation is responsible for this phenomenon.

(Dr. Henry E. Meleney, Nashville, Tenn.) It is very well known, of course, that in estivo-autumnal malaria, during the second half of the 48-hour cycle, the parasitized erythrocytes usually are much fewer in the peripheral circulation, and only ring forms and crescents are usually found. It is generally believed that this is due to the stickiness of the parasitized red cells in the capillaries. This happens even in the first cycle of the estivo-autumnal infection, and would, therefore, not be due to a specific agglutinin. Also, in some cases which I have seen, the larger venules in the brain and other organs have shown individual parasitized red cells forming a ring adjacent to the endothelium with nonparasitized red cells in the center of the lumen. This did not appear in the vessels which Dr. Cannon showed, but it certainly does in some cases.

(Dr. Cannon) With regard to Dr. Mudd's question as to whether this reaction within the capillaries is specific or nonspecific, I should say that the simplest explanation and the one that has usually been followed is that the reaction is nonspecific and due to injury of the parasitized cell. It seems to me, however, that if the reaction is nonspecific there should be nonparasitized cells entangled in the clumps of parasitized cells together with fibrin and leukocytes. The fact, however, that the emboli consist entirely of parasitized erythrocytes points quite clearly, it seems to me, to the idea that this clumping is due to a specific mechanism. Complete proof is impossible because we must depend solely on morphologic evidence. Inferential evidence, however, substantiates the idea of a specific effect in that in monkeys infected with *P. knowlesi* agglutinins have been demonstrated (Eaton and Coggeshall) and actual agglutination in the circulating blood has been shown (Knisely). The idea of a specific agglutination does not eliminate the possibility that nonspecific factors may also play a part, as Dr. Furth has mentioned in connection with leukemia.

A NONVIRULENT IRRADIATED RABIES VACCINE. Leslie T. Webster and (by invitation) J. Casals, New York, N. Y.

Abstract. The supernatant of a 1 to 5 per cent dog brain virus suspension exposed to ultraviolet light for 20 to 30 minutes becomes nonvirulent for mice and yet 0.1 cc. of this preparation immunizes them against a subsequent intramuscular injection of street virus. Thirty cc. quantities of this irradiated vaccine immunize beagle dogs.

THE HISTOPATHOLOGY OF HISTOPLASMOSIS IN MAN. Robert J. Parsons, Ann Arbor, Mich.

Abstract. The presentation consists of the demonstration of the gross and microscopic characteristics of the lesions found in the 4 cases of histoplasmosis which have been seen at the University Hospital. Complete autopsies were done in 3 of these cases and *Histoplasma capsulatum* was grown from the tissues of 2 of the cases.

mous concentration in the red pulp of the spleen, with innumerable parasitized erythrocytes being crowded together with no intermingling of leukocytes or of nonparasitized erythrocytes. Many capillaries in the myocardium, intestinal mucosa, pancreas and elsewhere were practically occluded by rows of parasitized erythrocytes without any intermingling of leukocytes or nonparasitized erythrocytes. Masses consisting entirely of parasitized erythrocytes were found in the lumen of pulmonary veins. Capillaries in the intestinal mucosa contained rows of parasitized erythrocytes with adjoining vessels filled with nonparasitized erythrocytes. The malpighian corpuscles in the spleen were markedly depleted and contained primitive undifferentiated mononuclear cells. There were marked fatty changes in the hepatic cells.

The pathologic changes in these 2 cases indicate that in the benign infection the parasites were easily disposed of by the main components of the reticulo-endothelial system, whereas in the malignant infection the parasites continued to develop despite the marked stimulation of lymphoid structures in the spleen and the efforts of the liver and spleen to remove them. The unequal distribution of clumps of parasitized erythrocytes, particularly as seen in the pulmonary veins and in the capillaries, suggests that this clumping may be the result of a specific antibody action. It is known that in malignant malaria of the monkey due to infection with *Plasmodium knowlesi*, agglutinins have been demonstrated which cause a clumping of parasitized red cells. The possibility that this also occurs in malignant human malaria is suggested by the fact that the masses of parasitized erythrocytes do not contain within them leukocytes or nonparasitized erythrocytes. It would seem that this latter condition would be present if the cause of the clumping is due, as has been usually assumed, to injury to the surface of the parasitized erythrocytes, with a resulting nonspecifically increased stickiness of the erythrocytic surface. Whether this tendency to embolic blockage of capillaries is due to specific or nonspecific factors, it would seem that the way to prevent this important complication of malignant malaria would be earlier and more effective drug therapy in order to keep to a minimum the relative numbers of parasitized erythrocytes.

Discussion

(Dr. George H. Whipple, Rochester, N. Y.) These pictures bring back the familiar appearances that we used to see constantly in the Canal Zone in 1907 and 1908. There, too, we were fortunate in being able to obtain material within an hour or two after death. The cases I remember particularly were those that came in in coma from the line, and were autopsied very shortly after death, and in almost all such cases a malignant type of malaria was found in which the capillaries of the brain cortex would be stuffed with these parasitized cells. The feeling of the physicians was that in this type of malaria with coma, nothing could be done by the largest doses of quinine intravenously, whereas if we could get them when they were beginning to show stupor they could be saved by heroic doses of quinine by vein.

(Dr. Stuart Mudd, Philadelphia, Pa.) I wonder whether it is possible to exclude the possibility that the change in the red blood cell might be non-specific; it is known that injured red cells are easily taken out of the circulation by the spleen, so that there must be some nonspecific change in the red cell surface as a result of injury.

(Dr. A. M. Pappenheimer, New York, N. Y.) I should like to ask whether the massive necrosis of the adrenal led to symptoms of Addison's disease.

(Dr. Otto Saphir, Chicago, Ill.) Is there any histological or morphological characteristic that distinguishes this fungous disease from other fungous granulomas?

(Dr. Parsons) In reply to the question as to symptoms of Addison's disease, the man whose picture I showed had a very low blood pressure, 82/60; there was no pigmentation, but we were not smart enough to study him for Addison's disease, in spite of the fact that it was the second case of complete caseation of the adrenals that we had seen in our 4 cases of histoplasmosis.

The morphological characteristics are very distinctive. The organism itself varies from 1 to 5 μ in diameter, or 6 μ , if you include the capsule. For the most part the large mononuclear cells, or histiocytes, or whatever one wants to call the large mononuclear phagocytes, are invaded. There is, so far as I know, no other fungous infection which produces this picture, with one exception, and that is the disease, Neapolitan farcy, seen in horses in the Mediterranean Basin. However, the fungus causing farcy is culturally very different from *Histoplasma capsulatum*. I did not mention that we had cultured *Histoplasma capsulatum* from 2 of our 4 cases. I am quite convinced that there may be some other organisms that have not been identified which might resemble this, but so far as I know the morphological features are quite characteristic. Final identification of the causative organism must be made on the basis of cultural studies.

THE HISTOPATHOLOGY OF EXPERIMENTAL AND SPONTANEOUS ENCEPHALOMYELITIS OF MICE (THEILER'S DISEASE). Peter K. Olitsky and (by invitation) R. Walter Schlesinger, New York, N.Y.

Abstract. Further evidence of the similarity between human poliomyelitis and spontaneous encephalomyelitis of albino mice (Theiler's disease) was obtained from an examination of more than 5,000 semi-serial sections from 30 brains and cords deriving from mice spontaneously attacked by the disease or experimentally infected with Theiler's virus, and by comparing them with sections obtained from albino mice infected with the Lansing strain of human poliomyelitis.

Lesions of two basic types are seen: (1) Mesodermal-glial; consisting of perivascular microglia and round cell infiltrations, perivascular gliosis, diffuse and focal gliosis and endothelial swelling and proliferation in smaller blood vessels; and (2) neuronal. Neuronal changes ranged from early stages of tigrolysis to necrosis, and finally to complete disappearance with resulting vacuolization of the stroma. Neuronophagia was less extensive than in human and experimental monkey poliomyelitis, and showed predominance of microglial elements rather than of polymorphonuclear leukocytes. Intracellular inclusion bodies, type B, were seen in early stages and were of the same type as those found in human and experimental poliomyelitis of the monkey. The distribution of lesions in Theiler's disease revealed no significant differences, whether the infection had been spontaneous or induced by intracerebral, intranasal, intralingual or intraperitoneal injection of virus. (The latter peripheral routes of inoculation were employed successfully in about 10 per cent of mice 2 weeks old.) In contrast to the positive findings in experimental poliomyelitis in monkeys, no lesions were found in the

The lesions demonstrated from 1 or more of our cases are: ulcerative granulomatous lesions in the skin, pharynx, tonsils, hypopharynx and larynx; chronic ulcers of the naris, ileum and colon; extensive confluent chronic lobular pneumonia due to the fungus; miliary tubercle-like lesions in the lungs of 2 cases (tubercle bacilli could not be stained); severe subacute granulomatous portal cirrhosis of the liver. Almost complete caseation necrosis of both adrenals was seen in 2 of the cases, and these were the cases in which miliary lesions were found in the lung. Parasitized large mononuclear phagocytes were seen in the bone marrow, kidney, spleen, thyroid and many lymph nodes. In 1 case the organisms were found in polymorphonuclear cells in the circulating blood.

The yeastlike form of *Histoplasma capsulatum*, or an organism morphologically indistinguishable from it, was found in all of the above-mentioned lesions with the exception of the miliary tubercles in the lungs of 1 of the cases.

Discussion

(Dr. Henry E. Meleney, Nashville, Tenn.) I think Dr. Parsons' paper is important in calling attention to this interesting fungous disease. When the previous paper on *Coccidioides* was being discussed, the question was raised about calcification in the old lesions of that infection. I have seen slides from many of the cases that have been reported as histoplasmosis and have never seen calcification in any of them. The association of this disease with tuberculosis is interesting. I have recently reported 2 cases of histoplasmosis associated with tuberculosis which occurred at the Vanderbilt University Hospital in Nashville. This paper is in press in the American Review of Tuberculosis. In both of these cases the *Histoplasma* organisms were limited to the lungs. In one a very extensive infection was present, a pneumonia such as was described by Dr. Parsons. In the other case there were cavities in the apices of both lungs, but only in the walls of these cavities were a few mononuclear cells found containing *Histoplasma*. In the first case there was miliary tuberculosis, with acid-fast bacilli found in the tubercles in the liver, spleen and lungs, so that I think the two diseases are often associated. On the other hand, there have been several cases in which tubercle-like lesions have been described which do not look exactly like tubercles, and yet neither *Histoplasma* nor acid-fast bacilli have been found in them. In one of our cases there were in the liver, associated with the necrotic lesions, some granules in large mononuclear phagocytes which looked as though they might be degenerated parasites. In a recent case from the Los Angeles General Hospital, which has not yet been published, the adrenals were involved and caseous, just as they were in the case reported by Dr. Parsons, and there were tubercle-like lesions throughout other organs without tubercle bacilli and also without *Histoplasma* organisms, although a few suspicious organisms were found. I should like to say a word of warning in connection with the reporting of this disease. I have recently had referred to me 3 cases of supposed histoplasmosis in which the lesions consisted of isolated nonulcerated skin nodules. Dr. Dawson of the Department of Pathology at Vanderbilt University agreed with me that they were not histoplasmosis, and he believed that at least 2 of the cases were adenomas of the sweat glands. In the large cells there were granules which looked a little like *Histoplasma*, but they had no capsule and no definite morphology.

therapy. I do not believe that the lesions described represent the complex of the various deficiency components, but that they are specifically due to a deficiency of nicotinic acid.

VITAMIN A DEFICIENCY AND THE CENTRAL NERVOUS SYSTEM. S. B. Wolbach and (by invitation) O. A. Bessey, Boston, Mass.

Abstract. Injury to the nervous system in vitamin A deficiency occurs only if the deficiency is established in very young, actively growing animals. We have been unable to produce lesions of the nervous system in white rats by vitamin A deficiency established after 10 to 12 weeks of normal growth. If the deficiency is established sufficiently early in life, neurological lesions invariably develop. Our procedure has been such as to prevent any considerable storage of vitamin A after birth and to place the rats on a vitamin A-free diet when weaned at 21 days of age. Ataxia and paralysis appear at about 50 days of age which is before the growth rate, as measured by weight increase, is appreciably changed. Administration of carotene at 42 days of age prevents the development of nerve lesions even though the diet is so restricted in amount that the treated animals grow less rapidly than litter mate controls continued on the vitamin A-deficient diet.

The neurological lesions in dogs, guinea pigs and rats are due to a disproportionate growth of the nervous system and skeleton. Our studies have been made chiefly upon white rats and confirmed in dogs and guinea pigs. In white rats the growth differential established by the deficiency is manifested in the gross by (1) overcrowding of the cranial cavity shown by distortion of the brain, dislocation toward the foramen magnum and multiple herniations of cerebrum and cerebellum into the venous sinuses of the dura at sites of arachnoidal villi; (2) overcrowding of the spinal canal with distortion of the spinal cord and herniations of nerve roots into intervertebral foramina and into bodies of vertebrae; (3) resorption of bone of the cranium and bodies of vertebrae in consequence of pressure. Early lesions of brain and nerve roots of the kind described in (1) and (2) have been found in rats killed before it was possible to elicit signs of functional disturbance.

A complete elucidation of the problem presented by these facts has not been achieved. The fact that vitamin A deficiency retards growth of bone must be taken into account. However, the study of rats whose growth rate has been more severely retarded by other deficiencies has shown normal relations of nervous system to skeleton. Litter-mate control work to date indicates that in vitamin A deficiency the nervous system grows at the same rate as in normal animals until that stage is reached where secondary starvation effects become operative. There is good evidence for believing that in vitamin A deficiency skeletal growth is retarded before that of the soft tissues in general.

Variations in distribution of the effects of the disproportionate growth in different species indicate that the order of initiation and rate of development of centers of ossification must be considered and suggest the possibility that a specific growth factor in bone is affected in vitamin A deficiency.

Histological studies have confirmed the conclusion that the nervous lesions in young animals, due to vitamin A deficiency, are mechanical in origin and due to the described disproportionate growth.

olfactory bulbs of intranasally infected mice. Following intracerebral inoculation, the first lesions were observed around the site of injection, whence they spread mainly periventricularly. Invariably the rostral parts of the brain and the cortex showed a predominance of mesodermal-glial lesions, while in the substantia nigra and throughout pontine structures and the medulla, neuronal changes were prominent. The anterior horn of the cord showed both types but, in contrast to the findings in experimental poliomyelitis in monkeys, apparently normal neurons were always present to a considerable number. No characteristic differences could be found between the lesions in the central nervous system of mice caused by the Lansing poliomyelitis strain and those induced by Theiler's virus.

DESCRIPTION OF SPECIMENS OF PELLAGRA. Robert A. Moore, Tom D. Spies, Zola Cooper (by invitation) and Harry Goldblatt, St. Louis, Mo., Birmingham, Ala., Cleveland, O.

Abstract. Two biopsies of the skin were examined from each of 15 patients who had pellagra and who had not received treatment. One biopsy was taken from an area of skin showing clinical evidence of pellagrous change; the other was taken from an area which was apparently unaffected. The skin from the unaffected areas showed little deviation from the normal. The epidermis, however, in all of the cases showed a slight hyperkeratosis, and in 9 of the 15 cases, focal areas of atrophy. The dermis was slightly edematous, and in 4 cases a mild infiltrate of lymphocytes was observed in the upper third of the corium. In 4 cases the sebaceous glands appeared atrophic. The biopsies taken from pellagrous lesions uniformly showed marked evidence of pathologic change. The epidermis in all cases was markedly hyperkeratotic, with patchy areas of parakeratosis and thickening of the glandular layer in 4 cases. There was in all cases also a marked acanthosis with lengthening and thinning of the rete pegs, and in some cases intracellular edema. In 3, foci of atrophy were present in the otherwise acanthotic epidermis. Vesicles which had been infiltrated with lymphocytes and polymorphonuclear leukocytes were found at the dermal-epidermal junction in 4 cases. In isolated cases there was an increase in pigment in the basal layer, and pigment could be found also in the spinous layer and even between the keratinized layers of the stratum corneum.

The dermis was in all cases edematous, and there was marked dilatation of the peripheral blood vessels. A moderate lymphocytic infiltrate was present in the upper third of the corium. In isolated cases the collagen fibers in the deep corium showed evidence of hyalinization and mucoid degeneration with necrosis of individual fibers. In 8 cases there was an apparent absence of sebaceous glands in the biopsies studied. This was thought noteworthy in view of the fact that the lesions were in an active stage and had not yet become atrophic.

Discussion

(Dr. Stuart W. Lippincott, Bethesda, Md.) I want to ask Dr. Moore whether he feels it is possible to separate these histological lesions in the skin in pellagra from those in patients who probably have an associated deficiency of riboflavin or pantothenic acid.

(Dr. Moore) I think this has been taken care of by Dr. Spies's observations that these areas of skin in every case responded to nicotinic acid

tail completely detached from the intestine. Two control operations, in which the tissues were handled but not cut, were performed and there were 3 unoperated control cats.

Disturbance in absorption of fats was manifest in the immediate rise in total lipid content of the stools from a normal level of 2 to 15 per cent to five or six times this amount. There was considerable fluctuation of prothrombin times. During the preoperative period these values varied within a range about 10 per cent above or below the average normal level. Following section of the ducts, fluctuations were enlarged to 48 per cent below and 116 per cent above the average normal values. After subtotal resection of the pancreas there was a marked prolongation of the prothrombin times, reaching an average of almost 100 per cent above the normal at one point. Again there was fluctuation in the readings and a gradual tendency toward restoration of the normal state. Whole blood clotting times were moderately elevated, the highest being 19 minutes following subtotal pancreatectomy. Bleeding times showed slight alteration.

The livers of the experimental cats were frozen and dried *in vacuo* promptly after death. The vitamin K content of the pulverized liver was determined by the Ansbacher curative method of bio-assay. The amount of vitamin was reduced after duct resection and markedly lowered following pancreatectomy. It was suggested that the loss of pancreatic lipase diminished the digestion of fats and absorption of the fat-soluble vitamin K and that production of prothrombin by the liver was accordingly impaired.

A VITAMIN B₁ DEFICIENCY OF FOXES PRODUCED BY FEEDING WHOLE FISH.

Robert G. Green, Minneapolis, Minn.

Abstract. A widespread disease in foxes and mink called Chastek paralysis and caused by the feeding of various kinds of fresh uncooked fish has been found to be a vitamin B₁ deficiency. The disease may be produced by adding 10 per cent ground whole carp to the ration, or by adding a mixture of heads, skin, tails, and fins and some scales. A ration containing the fillets of carp has not produced the disease in comparative tests. Chastek paralysis may be prevented by adding large amounts of thiamin to the fish ration. In its clinical and pathological manifestations, the disease is analogous to Wernicke's alcoholic encephalitis of man.

PATHOLOGICAL CHANGES IN THE MOUSE DUE TO PANTOTHENIC ACID DEFICIENCY. Stuart W. Lippincott and (by invitation) Harold P. Morris, Bethesda, Md.

Abstract. Pantothenic acid was first described in 1933 by Williams and associates as a bios or growth factor for yeast. During the last year it has been synthesized and proven to be necessary in the nutrition of the chick and rat. Because of its importance as a nutritional factor, its possible rôle in tumor metabolism *in vivo* in spontaneous mammary carcinoma of the C₃H mouse was investigated. Before proceeding to these experiments it was necessary first to establish whether the mouse required pantothenic acid and what the pathologic changes were in the deficient state. This report deals with the latter two phases.

In this strain of mice removal of pantothenic acid results in lack of growth in young animals, marked loss of weight in adults and inability of either group to survive for more than 8 weeks. During the course of the deficiency

THE DISTRIBUTION OF VITAMIN A IN OVARIES AND OVARIAN TUMORS. Hans Popper and Alex B. Ragins, Chicago, Ill.

Abstract. The fluorescence microscopic demonstration of vitamin A in tissues (Querner, Popper) was applied to the human ovary. One observes a dull green, very slowly fading fluorescence due to carotene, a bright green, quickly fading, and a fainter green, slowly fading fluorescence, both due to vitamin A and imparted by lipoids. In rat ovaries evidence exists for the specificity of this fluorescence for vitamin A; in the human ovary no other substance has as yet been found imparting a similar fluorescence. After the second postfetal month there appear fine droplets with vitamin A fluorescence in the stroma around the primordial follicles and in the maturing follicles a quickly fading fluorescence in the granulosa and a slowly fading one in the theca. The theca of the corpora atretica shows larger amounts of the fainter fluorescence.

After puberty the stroma around the primordial follicles is free of vitamin A fluorescence. In the granulosa of the graafian follicles fine droplets with vitamin A fluorescence are seen, whereas the theca cells contain the fainter green fluorescence. After ovulation the bright fluorescence of the granulosa imparted by fine droplets increases, especially in the stage of vascularization and maturity. A dull green carotene fluorescence appears in the cytoplasm of the cells. In involution a strong, but more slowly fading fluorescence is imparted by large droplets. Later on the vitamin A fluorescence disappears prior to the lipoids. The corpus luteum of pregnancy reveals vitamin A fluorescence in the theca cells, the granulosa showing only strong carotene fluorescence. The atretic follicles and the scattered theca cells impart a strong, slowly fading fluorescence. After fading, a brown fluorescence is visible which in older corpora atretica is seen immediately. The brown fluorescent material is a non-lipoid-soluble pigment, dark brown in visible light. After the climacterium no vitamin A fluorescence is visible. The physiological significance of the vitamin A fluorescence in the ovary is not established. The characteristic cyclic variations of the fluorescence suggest a relationship of the substance with vitamin A fluorescence to hormone production. The fluorescence may be considered as a morphological sign of hormonal activity.

Generally tumors impart vitamin A fluorescence when their mother tissue shows the fluorescence. Consequently granulosa cell tumors in places reveal the fluorescence of the luteinized granulosa cells, theca cell tumors that of the theca cells of the corpora atretica, xanthofibromas that of a cortical stroma with vitamin A fluorescence, arrhenoblastomas that of the Leydig cells, dysgerminomas that of the dysgerminoma of the testicle. The specific structures of fibromas, papillary cystomas, cystadenocarcinomas and fibroepitheliomas are free from fluorescence.

EFFECT OF PANCREATIC ACHYLIA ON VITAMIN K ABSORPTION AND PROTHROMBIN TIME. Edith E. Sproul and (by invitation) Elmer Key Sanders, New York, N.Y.

Abstract. Adult cats were deprived of external secretion of the pancreas: 7 were subjected to sectioning of the pancreatic ducts and separation of the head of the pancreas from the duodenum; 2 received a complete pancreatectomy, and in 6 about two thirds of the pancreas was removed, leaving the

(Dr. Stewart) How long had the stock been separated from the parent strain?

(Dr. Schenken) Since 1937.

EFFECTS OF LONG-CONTINUED INGESTION OF SODIUM PHOSPHATE UPON THE PARATHYROIDS, KIDNEYS AND BONES OF MATURE RATS. John A. Saxton, Jr., and (by invitation) Gordon H. Ellis, New York City and Ithaca, N.Y.

Abstract. Mature male rats were divided into two lots. One lot was fed an adequate diet containing 8 per cent of sodium metaphosphate. The other lot received a diet containing about the same quantity of sodium orthophosphate. These diets were continued for 7 months, or until the animals succumbed. X-ray examinations at intervals during life disclosed a gradual decalcification of bones and deposition of calcium in the kidneys. Urinary excretion of inorganic phosphorus was increased. Gross examination showed enlargement of the parathyroids from two to eight times that of the controls. The long bones appeared slightly thickened, but in some instances were more fragile than normal. Microscopic study showed hypertrophy and hyperplasia of parathyroid cells. Calcium deposits were present in the tubules of the kidneys, chiefly in the medulla. There were widespread metastatic calcium deposits in numerous organs. The long bones showed diffuse endosteal and periosteal new-bone formation with focal areas of osteoclasts and fibrosis. These observations suggest that some instances of human disease in which there are lesions of the osseous system and parathyroid hyperplasia may be a consequence of excessive ingestion of phosphates.

THE NEURO-INSULAR COMPLEX OF THE PANCREAS: ITS POSSIBLE RÔLE IN DIABETES. Louis C. Simard, Montreal, Canada.

Abstract. In 1925 Van Campenhout described in the pancreas of certain mammalian embryos a structure which he called the sympathico-insular complex. It consists in the intimate association of insular and nervous elements by migration into the nerves of cells arising from primitive pancreatic ducts. These complexes were considered to be a stage in the organogenesis of the pancreas as transitory structures which insured an ephemeral or embryonic function, but which were destined to disappear. The author endeavored to follow the fate of these complexes in the pancreas of the adult man (1937), and in several other adult mammals. The pancreatic glands on which this study is based were removed from man, pig, dog, cat, white porpoise, horse, ox, sheep, rabbit, guinea pig, raccoon, opossum and rat. In all these animals the complex, which should be called the neuro-insular complex, was found.

The structure of the complex is not uniform; rather it is characterized by a great variability as regards the proportion of epithelial and ganglion cells. The epithelial cells of the complex are insular cells. From the histophysiological point of view the neuro-insular complex of the pancreas might be considered as a typical neurocrine organ, being a part of the intrinsic nervous system of the gland. It might be an important organ in the autonomic regulation of insulinic secretion.

Total cross sections of the pancreases taken from persons who had died in diabetic coma were carefully examined by serial sections. In 1 case only two

there is depilation and scaling of the epidermis about the nose and over the scapular region and flanks. The animals become sensitive to touch, squeak in a characteristic manner, develop an awkward, stilted gait and finally partial paralysis of the hind legs. Histologically there is minimal fatty degeneration in heart, liver and kidney, as well as an hyperkeratotic, atrophic, desquamative dermatosis and myelin degeneration in the sciatic nerves and spinal cord, chiefly in the posterior funiculus. The gross and histologic changes are multiple manifestations of the widespread disturbance in normal tissue metabolism which occurs in the deficiency state after complete removal of pantothenic acid from the diet.

A QUANTITATIVE STUDY OF REVERSIBLE STABILIZATION OF POLYSACCHARIDE IN DYED CARTILAGE. George M. Hass, New York, N.Y.

Abstract. If fresh frozen sections of human epiphyseal cartilage are extracted at pH 11 for 24 hours at 5° C., a large percentage of chondroitin sulfuric acid is removed. If the sections are stained with crystal violet prior to extraction under these conditions, the polysaccharide is retained in the tissue in quantities which are proportional to the amount of dye bound in the tissue. If the tissues are stained with the dye and if the dye is subsequently extracted with alcohol, polysaccharide is set free at pH 11, provided the initial concentration of the dye in the tissue is not too great. Therefore, under the specified conditions of extraction, stabilization of chondroitin sulfuric acid in cartilage is almost completely reversible if a proper choice of concentration of dye in the tissue is made.

OCCURRENCE OF URINARY TRACT CALCULI IN INBRED STRAIN (C₃H) OF MICE TREATED WITH ESTROGEN: FURTHER OBSERVATIONS. John R. Schenken, Edward L. Burns and (by invitation) William M. McCord, New Orleans, La.

Abstract. Studies of estrogen-injected strain C₃H mice under controlled conditions revealed a high incidence of urinary tract calculi, particularly in males, as compared with a control group of animals. The injections, for which crystalline α -estradiol benzoate in sesame oil (progynon B) was used, were made in varying doses and for varying periods of time. The highest incidence was observed in males which had been treated for from 8 to 20 weeks and which then lived from 4.82 to 8.92 months longer. The calculi were of the "bone earth" type. Pathologic changes in the genito-urinary tract similar to those reported by other investigators were also observed. Apparently both metabolic and inflammatory factors may be responsible for the high incidence of calculi in estrogen-treated animals. The available evidence seemed to indicate that the metabolic factor was associated with a derangement of calcium metabolism. Other evidence indicated that epithelial hyperplasia with desquamation and urinary tract infection also played a part in the formation of calculi, particularly in male animals.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask whether this strain of C₃H mouse was obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.

(Dr. Schenken) We obtained the original strain from Bar Harbor; the animals were bred by brother-to-sister matings in New Orleans.

(Dr. von Haam) Two adenomas of the bronchial glands were found.

(Dr. W. C. Hueper, New York, N.Y.) Did you examine the brains of these animals? The fumes contain an appreciable amount of manganese and manganese is known to be toxic to the central nervous system.

(Dr. von Haam) We observed no changes in the brains in any of the animals.

(Dr. Samuel R. Haythorn, Pittsburgh, Pa.) The deposits in the lungs are almost identical with those obtained in experiments with bituminous coal smoke, and, as Dr. von Haam stated, are not at all similar to silj

CIRCUMSTANCES AND POSTMORTEM FINDINGS, ESPECIALLY SKIN
IN ACCIDENTAL ELECTROCUTION. Milton Helpern and (by
George Strassmann, New York, N.Y.

Abstract. This study is based on 144 cases of accidental electrocution which include the majority of such cases investigated by the Office of the Chief Medical Examiner from 1928 to 1938, and a number of cases observed abroad from 1924 to 1938. Of these 144 cases, 101 deaths were caused by high-tension currents of 600 v. or more, and 43 deaths by low-tension currents of 110 and 220 v., usually of alternating type. In the high-tension group there were 64 immediate deaths and 37 delayed deaths; of the latter, 23 persons died within 48 hours and 14 died in from 2 days to 2½ months after the accident. In the low-tension group there were 37 immediate deaths and 6 delayed deaths caused by ignition of clothing by burning insulation or short circuit sparks, rather than from the effect of electric shock. Necropsies were performed in 50 of the high-tension and in 29 of the low-tension cases. The circumstances were investigated and careful examinations made of the bodies in all of the 144 cases.

Twenty-six of the low-tension and 65 of the high-tension accidents occurred during work; 11 low-tension deaths in the home; 29 high-tension deaths as the result of trespassing and contact with high-tension wires and third rails, and 7 deaths from contact with fallen high-tension wires. In both low and high-tension groups, most victims were in the third and fourth decades of life, with a considerable number of children in the high-tension group. Of the victims, 135 were males. In 13 of the low-tension cases the victim cried out when the shock was received; in 24, the victim was found dead, the accident not having been witnessed.

Most of the accidents in both the low and high-tension groups occurred during the warm months of the year, with the peak incidence in July and August. The seasonal incidence was especially striking in the low-tension group, and is dependent upon conditions favorable for the passage of low-tension currents through the body. Hot, humid weather induces sweating, and the moist skin has a lessened resistance to the current. Moist soil and damp shoes and feet furnish excellent conditions for grounding. A considerable number of low-tension accidents occurred while the victim was washing out a boiler, standing in water holding an extension lamp in one wet hand and a hose in the other. There were several cases in which the victim touched an electric appliance or fixture while taking a bath.

The general pathologic findings are not characteristic. The necropsies in cases of immediate death revealed visceral congestion, pulmonary edema, fluid blood in the heart, at times petechial hemorrhages in the pleura, peri-

complexes could be found, in another, only one, and in the last 3, none was found. And yet, in the 5 cases selected, the islands of Langerhans showed no degenerative or hyaline lesions: 2 of them, on the contrary, had, as is frequently found, numerous hypertrophied islands. If one remembers (1937) that the complexes are quite abundant in man (7 in a series of 300 μ thick) one cannot but be impressed by the considerable reduction of the complexes in these pancreases.

This research should be continued on many other glands of diabetic patients, as it might throw some light on the pathogenesis of diabetes.

THE PATHOLOGY OF SHIELDED ARC WELDING. Emmerich von Haam and (by invitation) J. J. Groom, Columbus, O.

Abstract. The health hazards of the welding occupation are still under dispute among physicians and public health officials. Thus far almost every disease encountered in welders has been linked by some authority with the welding occupation. Because of the uncertainty of most of these statements, based solely upon clinical observations, we studied experimentally the lesions produced by exposure to welding fumes and welding gases. Rabbits, guinea pigs, rats and mice were exposed daily to the concentrated fumes and gases produced by three commonly used coated rods. The concentration of fumes was in most experiments far above the concentration levels commonly encountered in industry.

At the end of our experiments we were impressed by the low toxicity of the fumes and gases produced by the coated rods examined by us. The mixture of welding gases, which included a minimal amount of carbon monoxide and a varying amount of the "feared nitrous fumes," produced with some exceptions only irritative lesions of the trachea and bronchi. Never did the blood of the experimental animals show any significant rise of carbon monoxide following exposure. The welding fumes, whose composition varied with the type of rod, produced after prolonged exposure a heavy iron pigmentation of the lungs, which did not incapacitate in any way the respiratory function of the lung, such as is encountered in the common toxic dust diseases. Silicosis and asbestosis were not encountered. Common respiratory infections typical for the various species of rodents were observed more frequently during the period of exposure than during the period of observation. No significant changes in the blood counts, in the endocrine glands, the central nervous system, or in any other organs were observed. The animals frequently became pregnant and delivered healthy litters.

From the above results we felt justified in concluding that the welding products from coated rods of average composition, in the concentration commonly encountered in industry, do not possess toxicity as attributed to them by the largely speculative medical literature. For the occurrence of acute respiratory lesions an individual susceptibility and the existence of a previous or concurrent bacterial infection seem more important than the composition and concentration of the welding products.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask if there was observed any increase in pulmonary tumors in the treated as compared with the control mice.

bination of shock, toxemia and septic complications of the burns, or from the complications of associated blunt-force injuries. Electric shocks by low-tension currents either kill immediately or not at all, and are usually without sequelae.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) In respect to this most interesting paper, I have one minor technical question. Some papers in the literature make the dividing line between high and low voltages 1000 v. In this paper it is placed at 600 v. How is the dividing line determined?

(Dr. Helpert) The dividing line is quite arbitrary. Currents of 110 and 220 v., usually alternating, are utilized in the home and in industry, and are commonly referred to as low-tension currents. High-tension currents range from 600 v. into the thousands and are transmitted by underground cables, overhead high-tension wires and third rails. We have not encountered any current voltages between 220 and 600. High-tension shocks are always dangerous to life. Low-tension shocks are frequently sustained without injury, but under certain circumstances may be fatal.

COAGULATION TIME OF THE BLOOD AND MURAL VASCULAR LESIONS AS DETERMINANTS OF THROMBOSIS. R. Katzenstein (by invitation), M. C. Winternitz and (by invitation) E. Mylon, New Haven, Conn.

Abstract. The well known effects of tissue extracts on the clotting time of the blood and the production of thrombi is related to their content of thromboplastic substance. This varies considerably with different tissues. Lesions of the walls of the heart and blood vessels are not necessary for thrombosis. Moreover, such mural changes occur without superimposed thrombi if the stability of the blood from the standpoint of its coagulation is not advantageous. Location of thrombi after injection of tissue extracts indicates variation in the coagulation time of the blood in different parts of the vascular bed and suggests participation of different organs in effecting the balance of the coagulation time.

LIGATION OF THE PULMONARY ARTERY: ITS RELATION TO PULMONARY ATELECTASIS, PULMONARY CIRRHOSIS AND BRONCHIECTASIS. Joseph Tannenberg, New York City and Bedford Hills, N. Y.

Abstract. In experiments on rabbits the relationship of pulmonary atelectasis and bronchiectasis was studied in several series which are reported elsewhere with Pinner in greater detail. In these experiments the bronchus of one lung was obstructed from within, or from without by ligation at the bifurcation. In a parallel series pneumothorax was maintained on the side operated upon for the duration of the experiment (up to 7 months); in another series infectious material was deposited within the bronchus prior to ligation; in a final series the ligation was made so that only partial bronchial obstruction resulted. These results were obtained. 1. Pulmonary atelectasis without complications could be maintained for several months unless infections intervened. 2. The bronchi within the atelectatic lung were maximally constricted, in spite of the increased negative intrapleural pressure at the side of the atelectatic lung. 3. Shift of the mediastinal organs and of the over-inflated contralateral lung, and elevation of the diaphragm took up the thoracic space made vacant by the shrinkage of the atelectatic lung. 4.

cardium and conjunctiva. A stomach full of recently ingested food was often found.

The specific lesions are the current marks (Strommarke of Jellinek) and burns at the sites of contact, usually on the hands or fingers. In the low-tension group of 37 immediate deaths, these current marks and burns were absent in 11 cases, or in slightly less than one third. They were reported in all the others, although some were questionable in the absence of a microscopic examination and were usually found at the site of entrance of the current. In all but 1 of the high-tension group, current marks or burns were present, usually at the site of entrance and exit. In most cases of electrocution by low and high-tension currents there were marks and burns either on the left upper extremity, the left side of the trunk, the left leg or both legs, or on any of these sites combined with the right upper extremity, the distribution indicating that the path of the current traversed the heart and tending to support the theory that death results from ventricular fibrillation rather than from respiratory paralysis.

The gross appearance of the current marks is not too characteristic and their detection requires a careful search. They may appear as yellowish, slightly depressed, punctate, circular, elliptical or linear marks. There may be blisters occurring singly or in a row. Prolonged application of the current may produce charring. The microscopic picture is often characteristic. There is vacuolization or "honeycombing" (Schridde) of the stratum corneum, a splitting of the epidermis with threadlike elongation of the cells and nuclei of the deeper layers of the epidermis, the altered cells having a distorted whorled arrangement, or the layers of the epidermis and corium may be compressed and fused with alteration of the staining characteristics of the corium which appears lilac-colored in preparations stained with hematoxylin and eosin. Distortion and shrinkage of the cells and nuclei may be found in the hair follicles and sweat glands. Vesiculation of the epidermis is found in some cases and also charring of the skin and subcutaneous tissues. Microscopically, the current marks are distinguishable from antemortem abrasions and postmortem artefacts such as occur from vigorous attempts at artificial respiration or careless handling of the body. Blistering and separation of the epidermis resulting from postmortem putrefaction occur between the epidermis and the corium; the electric current splits the layers of the epidermis so that these marks can be distinguished from and are recognizable in the presence of putrefactive changes. The current marks do not represent a vital reaction, but are produced by the heat generated by passage of the current through the resistant skin. The marks may be indistinguishable from those produced by the application of hot objects to the skin and they may be simulated in this way.

In high-tension electrocutions, in addition to the ordinary current marks, there usually are extensive electrical burns due to ignition and incineration of the tissues, and also third degree burns produced by burning clothes ignited by the current. In high-tension cases with extensive burns, the ordinary current marks may be difficult to find.

Immediate death in high-tension electrocution may result from the combination of electric shock, extensive burns and blunt-force injuries sustained when the body falls or is thrown from a height at the time the shock is received. Delayed deaths from high-tension shocks may result from a com-

The mean age for the group was 44.1 years, and the average heart weight was 356 gm. A slight but significant positive correlation of 0.2670 was found between age and heart weight. It was not possible to exclude entirely the influence of hypertension as a factor accounting for this significant correlation, but it appeared that if hypertension rather than age were the influential factor, it did not account for more than 7 per cent of the variability in heart weight. Body weight and heart weight were significantly correlated in a positive direction, and this was independent of age. Height did not appear to contribute materially to the variability in the weight of the heart, except in so far as height affected body weight. The multiple correlation between heart weight and body weight and age was 0.6287. This gave the lowest standard error of estimate of any of the calculated correlation coefficients. The corresponding multiple regression equation was: estimated heart weight in grams = age in years $\div 3$ (body weight in kilograms) $\div 100$. It was concluded that a diagnosis of cardiac hypertrophy was justified when the observed heart weight exceeded by more than 77 gm. the estimated heart weight derived by the use of this regression equation.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) It is very interesting to observe the heart weights in this presentation, because it appears to show that the heart weight is distinctly greater than one would have expected from the figures published up to the present time. I would like to be informed in regard to the microscopic changes in some of the hearts. About 66 of the hearts were taken from patients who had died of acute causes, and these were presumably normal hearts. From my own experience, and from that of others, it seems quite clear that some of these hearts might show evidence of acute myocarditis, or at least of edema, and I think the edema can be seen in microscopic sections. If these 66 cases had not been included I would have liked the results much better, but this edema which might have been present in the myocardium might outbalance the results of the other cases.

(Dr. E. T. Bell, Minneapolis, Minn.) I am inclined to put much more stress on hypertension as explaining the age increase in the heart weight than Dr. Rosahn does. It is well established that about 40 per cent of males over 50 years of age have a blood pressure of 150/90 or higher, and if you take out all hypertensives most of that age increase disappears.

(Dr. Rosahn) As I said in the presentation, I did not personally view the microscopic preparations of the hearts that constituted this survey. However, as a routine in the Department of Pathology, sections are taken from the heart and studied in conference and if no abnormality is present, the heart is considered to be a normal heart. I can say none of the cases had acute myocarditis. There is a possibility—I do not know how probable it is—that some of them did show some degree of edema. I will be very happy in further presentations on this subject to take that suggestion into consideration and include only those cases in which no edema can be shown microscopically.

With regard to Dr. Bell's question of hypertension being the factor involved rather than age in the increase in heart weight, it is not possible, of course, to exclude hypertension as the primary factor. But should one ascribe to hypertension alone all of the variability which is the result of age, then

Bronchiectases from cylindrical to most severe saccular forms occurred as a consequence of infections producing purulent bronchitis in the obstructed lungs. 5. Under such conditions *pneumothorax* on the obstructed side did not prevent the formation of bronchiectases. 6. Partial obstruction of the main bronchus led to emphysema, not to bronchiectasis, if infection was prevented.

Ligation of the pulmonary artery on one side in a series of rabbits which were kept alive for a period up to 5 to 6 months had these results: Not simple atelectasis, but hemorrhagic infiltrations and necroses of varying extent were the early changes. If the animals survived the first few days, organization of the hemorrhagic necroses and subsequent fibrotic shrinkage of the involved areas developed. This quite different condition, nevertheless, produced practically the same roentgenological picture as pulmonary atelectasis, the same dense X-ray shadow of the involved lung, and shift of the mediastinal organs and the contralateral lung to the affected side.

After ligation of the pulmonary artery shrinkage of the lung takes place while the atmospheric air has free access to the bronchial tree. In this respect the conditions resemble closely those which exist in man when bronchiectasis develops. The results obtained fully confirmed those obtained after bronchial obstruction. If there was no complication by infection, no bronchiectasis developed despite shrinkage of the lung to a fraction of its normal volume. When spontaneous infection of the bronchial tree intervened, or upon artificial infection with a bovine strain of tubercle bacilli, bronchiectases of various sizes occurred; in the latter case, however, only when tuberculous foci had established themselves in a bronchial wall causing destruction of the bronchial musculature.

The pleural space made vacant by the shrinkage of the lungs was filled by shift of the mediastinal organs, over-inflation of the contralateral lung, and over-inflation and emphysema of those parts of the isolateral lung which were preserved.

Discussion

(Dr. Max Pinner, Bedford Hills, N. Y.) I think there is one important clinical and roentgenologic conclusion to be deduced from these experiments. The roentgenologic criteria for the diagnosis of pulmonary atelectasis; namely, the ground-glass appearance of the lung, the shift of mediastinum and diaphragm, and so on, are taken in general as proving the presence of atelectasis. However, it is shown by these experiments that other pulmonary conditions may produce identical roentgenologic appearances. This is true for the lung which becomes shrunken and organized and completely fibrotic following ligation of the pulmonary artery. It is equally true for the lung which is shrunken but infected and contains large bronchiectases. As I said in the beginning, the current criteria for pulmonary atelectasis are not sufficiently differentiating for uncomplicated atelectasis, but apply to a number of other pulmonary diseases as well.

WEIGHT OF THE NORMAL HEART IN ADULT MALES. Paul D. Rosahn, New Haven, Conn.

Abstract. A group of 187 males 20 years of age and over, with no cardiovascular disease at autopsy, dying either from trauma or from an acute disease requiring not more than 2 weeks' hospitalization, was studied to determine the influence of age, height and body weight upon the weight of the heart.

small arteries of the atrophic kidneys of otherwise nonarteriosclerotic persons with chronic Bright's disease. Although it remains to be demonstrated whether or not obliterative renal arterial change may be caused by reduced blood flow through the kidneys, evidence has been presented that similar changes in other vessels are often adaptive and occur as a result of reduced circulation.

Discussion

(Dr. Joseph Tannenbergh, Bedford Hills, N. Y.) I would like to ask Dr. Moritz if there were any changes in the nutritional vessels or vasa vasorum of the isolated arterial segment, when proliferation of the intima was observed?

(Dr. Moritz) The only fixed tissue changes seen in isolated segments were in those in which thrombosis occurred. Up to 43 days no intimal changes were noted in isolated segments which had been washed clean so that there was no thrombus.

THE PATHOLOGY OF ARTHRITIS DEFORMANS. S. A. Goldberg, Newark, N. J.

Abstract. In a study of joints from surgical and autopsy material, lesions have been encountered that appear to divide arthritis deformans into two main groups: those in which the lesions are possibly due to an infective agent and those in which the lesions are possibly due to trauma. This trauma may be extrinsic or due to the wear and tear of advancing age. There are cases of arthritis deformans in which there appears to be a combination of these two groups of lesions.

In the first group the synovial membrane is thickened by villous or papillary growths of fibrovascular tissue containing perivascular lymphocytic or plasma cell infiltration. The articular cartilage may be completely replaced by granulation tissue, the areas of remaining cartilage degenerated and covered by vascular tissue containing lymphocytic infiltration. These changes do not result in true ankylosis. Inability to move the joint is due to pain or to distortion of the articular surfaces. The subchondral bone is atrophied, possibly due to disuse. In the early stages there is a synovial pannus in the joint producing erosion of the articular cartilage by direct dissolution, suggestive of enzyme action.

In a study of early lesions of arthritides in young animals, (Equidae and Bovidae) it was seen that the earliest pannus formation is a thin vascular film emanating from the synovial membrane, from around the interosseous ligament, or from the subchondral marrow of a preëxisting articular erosion. The erosions may also be formed by penetration of the articular cartilage by fibrovascular tissue from the subchondral bone, as shown by Bauer. In the early lesions the cartilage at first shows a change in the staining reaction. Normally cartilage takes the alkaline stain and appears blue. In these lesions the cartilage takes the acid stain and appears pink. This indicates a change in the pH of the cartilage before the cartilage completely disappears. Eventually portions of the subchondral bone also become eroded and replaced by fibrovascular tissue. Ankylosis may follow these erosions or the vascular pannus, even without complete destruction of the opposing articular cartilages.

In the second group the articular cartilage is thickened by proliferation of cell nests that later undergo degeneration and take the form of fibrils per-

hypertension, in this series at least, did not contribute more than 7 per cent to the total variability in heart weight, and that is the maximum contribution of age alone. I do not believe that hypertension *per se* is the important factor involved. As a matter of fact, certain of these cases were included which showed some degree of atrophy.

THE RELATION OF THE "MYOCARDIAL RETICULOCYTE" TO THE ASCHOFF NODULE. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. The "Anitschkow myocyte" or myocardial reticulocyte of Ehrlich and Lapan is a cell with a peculiar morphology, characterized by having an elongated, serrated chromatin bar within the nucleus with fibrillar extensions toward and to the nuclear membrane. It is found only in the heart and heart valves. This cell responds in rheumatic inflammation by proliferating and by taking on more cytoplasm which stains darkly. It is often the chief cell found in Aschoff nodules and appears always to be present in a greater or less degree. The nuclei in the giant cells in the nodules have the typical morphology. The response of this cell is not characteristic of rheumatic inflammation, for the cell is not found in rheumatic subcutaneous nodules and is found in nonspecific inflammation in the heart. The presence of this peculiar cell in rheumatic inflammation may help to explain the term "typical Aschoff nodule."

Discussion

(Dr. Paul Klemperer, New York, N. Y.) Dr. Ehrlich, working in our laboratory, has worked along the same line. I should not like to call the cell a "histiocyte"; it is a multipotent cell and we used to refer to it as the "mesenchymal cell" of the cardiac skeleton. I have seen, and I wonder whether Dr. Clawson agrees with me in this, a transition of this type of cell into fibroblasts. The cell is stimulated in general infections without any conspicuous lesion of the heart. It is more frequent in children than in adults.

(Dr. Clawson) I have seen a cell of this type apparently changing to a fibroblast.

THE PATHOGENESIS OF ARTERIAL ATROPHY. Alan R. Moritz, Boston, Mass.

Abstract. Varying degrees of local circulatory stasis were induced in segments of carotid and femoral arteries of dogs and the subsequent reactive vascular changes were found to be similar to those which occurred in the splenic arteries of infants and children following splenectomy. Three types of adaptive vascular change were observed as a result of diminished blood flow. In some arteries contraction unaccompanied by fixed tissue proliferation comprised the full extent of the reaction. In others intimal hyperplasia was superimposed upon the vascular contraction. There was evidence that intimal hyperplasia may occur without antecedent mural thrombosis. New elastic fibers were formed eventually in the hyperplastic intima. In still others the obstructed segments of vessels were occluded by thrombosis.

These experimental involutional changes, if they may be designated as such, are similar in some respects to those which occur in the intra-abdominal portions of the umbilical arteries of young infants, in the uterine arteries of young women during the postpartum period, in the cortical ovarian arteries of women during the catamenial period of life and in the intermediate and

HYPERTROPHIC PULMONARY OSTEO-ARTHROPATHY. A PATHOLOGIC STUDY OF SIX CASES. Edward A. Gall and Granville A. Bennett, Boston, Mass.

Abstract. Although clubbing of the fingers, one of the cardinal manifestations of hypertrophic pulmonary osteo-arthropathy, has been known since antiquity, it has only been comparatively recently that the wider distribution of the malady has been appreciated. It is our purpose to discuss the histopathology of the associated lesions, a phase of the subject which has received remarkably little attention. Material for this study was obtained by biopsy or necropsy from 6 patients who had, in addition to the osteo-arthropathy, in 4 instances pulmonary neoplasm, in 1 pulmonary emphysema, and in another, congenital heart disease. All had clubbed fingers and in 4 patients for whom roentgenograms of long bones were made, there was evidence of subperiosteal ossification to a varying degree in the tibia, fibula, femur, radius, ulna, metatarsals, metacarpals and proximal phalanges.

Histologic study showed that the periosteal lesion followed a well defined developmental pattern. Initially there was division of the periosteum into an outer fibrous zone in which an extensive lymphocytic infiltration was apparent and an inner edematous cambium layer. The swollen cells within the latter apparently made possible a deposit of osteoid upon the subjacent cortical bone. As the amount of osteoid increased, the deposits were arranged in a columnar fashion perpendicular to the bone surface. Calcification occurred in the deeper and older portion of the osteoid and fusion with the cortex took place. Osteogenesis was also noted to a marked degree in tendons at points of insertion. Continued ossification permitted the development of cancellous bony sheaths irregularly encasing the shafts of the involved bones. Intermittent periods of activity resulted in this sheath becoming laminated. Ultimately lacunar resorption of the original cortical compacta caused this also to become cancellous in structure and indistinguishable from the overlying subperiosteal bone. The thickness of the cortex thus became considerably greater than normal but it was exceedingly porous.

Specimens of clubbed digits from 4 cases exhibited minimal or no subperiosteal bone formation in the terminal phalanges. The clubbing evidently had resulted largely from edema and inflammatory infiltration of the soft tissues in this region. Joint tissues removed from all of the 6 cases showed in 4, slight to moderate edema and lymphocytic infiltration of the synovia and varying degrees of degenerative change in articular cartilage. None of these lesions could be considered specific.

It is concluded that hypertrophic pulmonary osteo-arthropathy in its fully developed form may be properly defined as that syndrome occurring as a sequela to a major visceral disease, usually intrathoracic in location, which is characterized by clubbing of the digits, ossifying periostitis mainly of long bones, and is frequently associated with joint manifestations. The sequence of events in the periosteal lesion has been described.

SPECIFIC THERAPEUTIC SHOCK—THE HUGH YOUNG REACTION. Ward J. MacNeal, New York, N.Y.

Abstract. When in the course of an established generalized bacterial infection, in particular with such organisms as staphylococci, colon bacilli or hemolytic streptococci, there is introduced into the blood stream an adequate

pendicular to the articular surface. The subchondral bone does not appear to be involved in this process. This is interpreted by Parker and Keiffer as an anatomical condition due to advancing age, and by Callender and others as a degenerative arthritis. In this series of cases, one of which was in a patient 5 years of age, the lesions were associated with definite trauma. In the group of older patients the lesions in the articular cartilage were possibly caused by circulatory disturbance produced by endarteritis obliterans of the anterior and posterior tibial arteries. All the patients complained of pain. This was probably due to stretching of the synovial membrane since nerve endings have not been demonstrated in articular cartilage or in subchondral bone. This condition is extremely common in man and animals as pointed out by Callender and Kelser.

THE PATHOLOGY OF THE JOINT LESIONS IN PATIENTS WITH PSORIASIS AND CHRONIC ARTHRITIS. Granville A. Bennett and (by invitation) J. Wallace Zeller and Walter Bauer, Boston, Mass.

Abstract. Included among 31 autopsied, and approximately 100 surgically treated, cases of arthritis of the rheumatoid type from whom articular tissues had been obtained for study were 7 having psoriasis. In 5 of the 7 cases the clinical and roentgenological findings and the pathological changes in the joints were indistinguishable from those of rheumatoid arthritis. The sixth case had, in addition to a widespread ankylosing arthritis, pronounced psoriatic involvement of the fingernails with marked arthritis of some of the terminal phalangeal joints. Although no tissue was obtained from these joints, the resected metatarsal phalangeal joints showed changes that were identical with those observed in rheumatoid arthritis.

The remaining case (a man 68 years of age) was of special interest. At 24 years of age he first noted arthritis in one terminal phalangeal joint. During the last 22 years of life he had complained of progressive arthritis of the terminal phalangeal joints of the hands and feet. He had been aware of the presence of psoriasis for 29 years. Psoriatic lesions of the nails were present. At autopsy it was possible to examine all of the joints of one hand and both feet and the majority of the other articulations. No lesions other than those of degenerative arthritis were found in any articulations except those of the fingers and toes. The majority of the terminal articulations were entirely destroyed and replaced by dense hyalinized scar tissue in which only minimal traces of inflammation could be detected. Extremely marked overgrowth of bone had occurred around the margins of the proximal ends of the terminal phalanges. The distal ends of the middle phalanges had undergone pronounced resorption. Bone atrophy, however, was not evident. The observed changes in these joints were unlike those of any usual form of chronic deforming arthritis. The dissimilarity between these joint lesions and those of rheumatoid arthritis was sufficiently great to suggest important differences in pathogenesis, if not in etiology. We have, however, been unable to eliminate the possibility that even these unfamiliar joint lesions may represent a rare form of rheumatoid arthritis.

Until additional information has been obtained, it is our belief that if the term "psoriatic arthritis" is to be used to designate a form of joint disease, its use should be restricted to cases such as this in which the arthritis is limited to the terminal digital joints.

5 seconds' duration. Lower values produce temporary disturbance, the duration of which is mathematically correlated to amperage and time. These experimental findings are applied to the interpretation of the clinico-pathological findings in accidental cases in man.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) This is a contribution of great significance. The description of the gel has included soft tissues. What information is there as to the passage of the current through bones?

(Dr. Alexander) We have found that bone passed the same amount of current as all other tissues, presumably because of the vascular bed which pervades living bone as completely as any other tissue.

(Dr. Alan R. Moritz, Boston, Mass.) I should be interested to hear a little more explanation of how the cows were killed.

(Mr. Weeks) The transformer on the pole broke down in very rainy weather; the current went down the wet pole and was not grounded in that area, because the underlying structure of the ground was sand and rock; therefore it flowed through the earth for a distance of three-quarters of a mile, and the cattle got enough current up their legs to cause their death.

(Dr. Alexander) The fact that the power company which owned the transformer paid damages for the death of those cattle is a point of confirmatory evidence. This was done after a very careful electro-technical investigation of the accident.

(Mr. Weeks) I might also point out that they were also all blooded cattle.

(Dr. Moritz) I suppose the wire fence might have had something to do with it.

(Mr. Weeks) Yes, the wire fence played a part in the picture. The fence was fastened to the pole which had held the faulty transformer, and the current went up through the fence. It is possible that one of the cows was killed by touching the fence, but the others were back of the fence and therefore they must have been killed by the current through the earth. It is not an unknown phenomenon. Horses have been killed by touching a third rail.

(Dr. Moritz) If an electric current tends to follow the pathway of least resistance, why did it deviate from its course to pass through the body of the cow?

(Mr. Weeks) The key to that situation is that the potential gradient is steepest near the point where it leaks out. If we apply that, we will have a high voltage near the pole, tapering off as it spreads away, and these cattle were near the pole. Therefore they were at the point nearest the greatest potential gradient.

(Dr. Jacob Werne, Jamaica, N. Y.) Were these cattle examined for lesions?

(Mr. Weeks) They were not.

THE LEUKOCYTIC RESPONSE IN EXPERIMENTAL SHOCK. Theodore J. Curphey and (by invitation) Eric Ponder, Mineola, N. Y.

Abstract. The polynuclear count (modified Arneth count) of Cooke is recognized to be a sensitive indicator of bone marrow activity, and it is known that a deflection of the count can be produced in rabbits under urethane anesthesia by crushing of muscle or bone, by irradiating with X-rays or

amount of suitable therapeutic agent, such as mercurochrome, antibacterial serum or bacteriophage, one may observe the production of a definite and often severe chill associated with and followed by a rise in temperature of 1° to 8° F., quickly succeeded by marked diaphoresis and defervescence and continued clinical improvement. This phenomenon, called by us (Sheplar, Adele E.; Spence, Martha Jane, and MacNeal, Ward J.: Serum therapy for infections with streptococci. *Arch. Surgery*, 1938, 37, 772) the Hugh Young reaction, resembles somewhat the paroxysm of early tertian malaria. It is evidently associated with an active phagocytosis of the bacteria in the blood and with a reduction in number of the circulating neutrophilic leukocytes which seem to phagocytize the injured bacteria and to be themselves, in turn, phagocytized by endothelial cells in the spleen, liver, lymph nodes and bone marrow.

Discussion

(Dr. Eugene L. Opie, New York, N.Y.) Does this reaction resemble that which is induced by sanocrysin and other gold salts used in the treatment of tuberculosis? Sometimes following injection there was elevation of temperature of from 3° to 5° F. It was assumed for a time that the reaction could be controlled by antisera against products of tubercle bacilli, but this relation was not demonstrable.

(Dr. MacNeal) I do not quite understand the question, but I do not believe I could answer it, anyway. It was a question of the injection of something in the treatment of tuberculosis, with a rise in temperature.

(Dr. Opie) In some instances about 3 hours after injection of gold salts there was elevation of temperature.

(Dr. MacNeal) Undoubtedly it is a related reaction. I do not mean to say that this Hugh Young reaction has not many things in common with other reactions, but there is this peculiarity, that the substance injected into the body is known to have a deleterious effect, *in vitro*, on the microörganism present in the blood stream, or in a very active lesion in contact with circulating blood; and secondly, when we inject it in adequate quantity we get a sharp chill and a sharp rise in temperature, and a subsequent fall and improvement in the condition of the patient. Those are the criteria for this peculiar reaction. In the instance mentioned by Dr. Opie, the agent injected does not give this reaction for about 3 hours. The microbe is not present in the blood stream, either, and it seems to me, therefore, that it may be a related rather than an identical reaction.

ELECTRIC SHOCK: IMPORTANCE OF PATH, DISTRIBUTION AND DENSITY OF CURRENT IN DETERMINING SYMPTOMS AND PATHOLOGY. Leo Alexander and (by invitation) Arthur W. Weeks, Boston, Mass.

Abstract. A clinico-pathological and experimental study on electric shock was presented. Experimental study shows that electric current passes through the animal body as though it were passing through a structureless gel, always choosing the shortest path from contact to contact without deflection by anatomical landmarks. Living bone carries a similar amount of current, presumably because of the vascular bed which pervades living bone as completely as most other tissues of the animal body. A critical level for lasting disturbance with definite morphologic alteration of nerve tissue was found to be at 30 milliamperes per 3 mm. of nerve diameter for shocks of

with nucleotides. In the experiment of the above investigators, the femoral region is crushed. No doubt there is extreme local damage, and it therefore seems to me conceivable that the leukocytosis is referable to the liberation of the same type of factor as the leukocytosis-promoting factor. It would therefore be of interest to see whether one can isolate from such a damaged tissue this active globulin.

(Dr. Ponder) We are quite in agreement with you on the nature of the substance. In 1930 we pointed out that it is a protein; we did not go so far as to show it was a globulin. I am familiar with your work, and from my experience am able to corroborate such parts of it as I have tried. I hope I have not given the impression that because I used nucleic acid as the standard in the assays I think the substance produced in the animal is nucleic acid. I used to use thyroxin, but nucleic acid is more convenient, and that is the only reason I use it.

(Dr. Robert A. Moore, St. Louis, Mo.) May I ask Dr. Ponder if there was any difference in the response of individual animals of the same species? We have secured some evidence in mice that the response of the bone marrow to either Dr. Menkin's material or to nucleic acid is a part of the genetic constitution of that animal, and that some animals respond by a leukocytosis and some give no response at all.

(Dr. Ponder) As regards the mouse, we have not found it possible to use this method of assay, because a mouse's polymorphonuclears are peculiarly complicated, and very difficult to resolve into their parts. As regards the genetic constitution, I should not be at all surprised. Different guinea pigs give different responses and it is a source of some trouble; but our experiments are not extensive enough, in that our colony is not large enough, for us to be able to say whether there is any genetic effect. It would not matter, I imagine, in an individual assay.

CERTAIN PHYSIOLOGICAL DIFFERENCES BETWEEN SHOCK AND HEMORRHAGE.

David R. Morgan, Marshall M. Lieber and (by invitation) Donald McGrew, Philadelphia, Pa.

Abstract. Comparison between animals in deep shock (intraperitoneal muscle implantation) and those dying from repeated hemorrhages shows that in the dogs with shock there develops a hemoconcentration of 36 to 40 per cent with an increase in the hemoglobin values, specific gravity, red blood cell count, white blood cell count, prolongation in the coagulation time and a marked fall in the sedimentation rate. There is a decrease in plasma volume, in plasma protein and in CO_2 content, an increase in the blood nonprotein nitrogen, and an inconstant blood sugar rise. Conversely, the blood of dogs after hemorrhage shows a distinct and immediate hemodilution with a decrease in red blood cell count, hemoglobin and specific gravity, a slowing of the coagulation time and an increase in the sedimentation rate. There is an increase in the plasma volume, the plasma protein, the plasma CO_2 and blood nonprotein nitrogen. The blood sugar shows an agonal rise. The lymph flow is increased in shock and decreased after hemorrhage. The parenchymatous tissues show a greater water content in shock than after hemorrhage. The urine in shock shows albumin, red blood cells, casts, bile salts and pigments and occasionally urinobilinogen. The urinary findings after hemorrhage are negative. There is a marked difference in the response

ultraviolet light in high intensities, or by the injection of extracts of macerated tissues. The stimulating agent in the extracts seems to be a protein. The deflection of the polynuclear count, conveniently expressed as a change in the figure for its mean, occurs within a few hours and passes off gradually, from 1 to 3 weeks being required for a return to the original steady state. Attempts have been made to assay the effect against that of a standard marrow stimulant such as nucleic acid injected under the skin of the back, but the deflection and return are too slow in the rabbit to allow this to be done.

In the guinea pig the polymorphs are much more complex, some of the cells having as many as ten nuclear lobes. This gives a right-handed polynuclear count with a mean of about 5.0 as compared with 2.0 to 2.5 in the rabbit. The marrow response to a standard injection (e.g., 1 mg.) of nucleic acid is very short and transient, the maximum deflection occurring in about 2 hours and recovery being nearly complete after 5 hours. This makes it practicable to standardize the response of each experimental guinea pig to various amounts of nucleic acid, and such standardized animals may be used as test animals by receiving injections of the plasma of other guinea pigs which have been put into shock by the standard methods of bone-crushing, burning, etc. In the injured animals the polynuclear count becomes deflected to the left and the mean falls until it reaches about half its original value at the time when the animal shows circulatory collapse with unquestionable hemoconcentration. If about 1 cc. of the plasma of this animal is injected into a calibrated test guinea pig, the polynuclear mean of the latter falls, and the minimum, reached in about 2 hours, can be compared with the known response to nucleic acid and assayed in this manner. The test guinea pig, after receiving the plasma from the animal in shock, shows striking changes in behavior, accompanied by coldness of the feet and ears. As the polynuclear count is deflected, the polymorphs themselves show an altered cytology, the nucleus becoming foggy and ill-defined. This disappears as the count returns to its original state, which it does after a few hours, with minor irregularities sometimes showing themselves for 2 or 3 days.

The deflection of the polynuclear count is accompanied, generally speaking, by an increase in the total number of polymorphs per cmm., but the total count is so variable that we have discontinued using it in quantitative work.

Discussion

(Dr. Valy Menkin, Boston, Mass.) This paper is extremely interesting to me, because 2 years ago we were able to identify a leukocytosis-promoting factor in inflammatory exudates, particularly as encountered in animals that had a leukocytosis concomitant with inflammation. This factor has been shown to be a globulin, or at least to be associated with it. It has also been found in dog and rabbit exudates. Reifenstein of Syracuse has recently confirmed its finding in rabbit exudates. In unpublished work I have recovered it recently from human exudates. In other words, this factor can be identified in a number of different exudates produced by a variety of irritants in several animal forms. The evidence does not seem to point toward nucleic acid as the active factor encountered. We have in our studies been able to dispose of the nucleoproteins. In general the reaction elicited by the leukocytosis-promoting factor (LPF) is much more rapid than that obtained

(Dr. Virgil H. Moon, Philadelphia, Pa.) It has been one of the problems of shock that so many complicating factors are introduced. Hence in all of our work we try to avoid complicating factors and to test the effects of shock, so far as possible, uncomplicated by other agents. This work is a preliminary report on a comparison of shock and hemorrhage as separate items. There were only one or two points at which a complicating factor was introduced, and that was done intentionally. In dogs in shock we noted that only a small amount of hemorrhage was necessary to produce a fatal effect when the dog's circulatory deficiency had reached a critical level, whereas after hemorrhages it required an enormous loss of blood to bring the dog to a lethal point. I hope all present will realize we are trying to compare two separate items and to avoid complicating factors when possible.

STUDIES ON THE BLOOD HISTAMINE IN RABBITS DURING HEMORRHAGE, SHOCK PRODUCED BY MANIPULATION OF THE INTESTINES, AND FOLLOWING THE SUBCUTANEOUS INJECTION OF HISTAMINE. Bram Rose and J. S. L. Browne (by invitation), Montreal, Canada.

Abstract. The histamine content of the whole blood and of the plasma was studied in rabbits during hemorrhage, shock produced by manipulation of the intestines during ether anesthesia, and following the subcutaneous injection of histamine. The results obtained show that the general pattern of change, namely a decrease in the histamine content of the whole blood and an increase in that of the plasma, is the same in these three conditions. The degree of decrease in the histamine content of the whole blood is similar in all three, but there is a difference in time relationships. For example, in the case of intestinal trauma, the onset of shock is slower, and the decrease in the whole-blood histamine level is spread over a period of from 6 to 12 hours. In the case of hemorrhage, or the subcutaneous injection of histamine, the decrease is more rapid, coming on within an hour, and reaching a maximum within 2 hours. The increase in the histamine content of the plasma is greatest in those animals which were given a subcutaneous injection of histamine and less in that of the animals subjected to the effects of hemorrhage and intestinal manipulation.

From these findings, it thus appears that histamine may be in some way related to the shock syndrome in the rabbit, and to hemorrhage. It is as yet difficult to interpret whether the increase in the histamine content of the plasma or the decrease in the histamine content of the whole blood is the more significant. In this connection, however, it is interesting to note that anaphylactic shock is correlated with a rapid and marked decrease of the whole-blood histamine level in the rabbit (Rose and Weil, 1939), that the histamine content of the blood of patients in shock due to trauma or surgical interference is markedly decreased (Rose and Browne, 1940), and that the symptoms of histamine intoxication in patients following the subcutaneous injection of histamine may be correlated with decrease in the histamine content of the blood (Rose, 1940).

Discussion

(Dr. Virgil H. Moon, Philadelphia, Pa.) Dr. Dragstedt has demonstrated in anaphylactic shock that there is a marked increase in the histamine content of the blood, and in the cases of serious shock reported here there was

to hemorrhage at the critical blood pressure level of 80 mm. of mercury. Dogs with shock could be brought to the death point with a single small hemorrhage, whereas dogs following hemorrhage withstood subsequent bleeding until the production of exemia.

Discussion

(Dr. Max B. Lurie, Philadelphia, Pa.) May I ask how the blood volume was determined?

(Dr. McGrew) In this series we did not determine the blood volume. In this current series, in trying to differentiate between shock and hemorrhage, no mention was made of the blood volume.

(Dr. Lurie) I mean the plasma volume.

(Dr. McGrew) We used the results of the work that has been done previously on normal dogs by Gibson at Harvard, in which he took normal dogs and correlated the plasma volume, the hematocrit reading and the hemoglobin. These figures, plus the direct hematocrit determination, made by taking the blood, citrating it and centrifuging it rapidly, which gives a rather gross determination of the plasma volume and also the amount of hemoglobin increase and the red cell content, give a fairly accurate indication as to the amount of plasma volume. The plasma protein we determined by the chemical analysis of the plasma.

(Dr. Stuart Mudd, Philadelphia, Pa.) I should like to point out that burns are apt to accompany conditions of shock and hemorrhage, and I want to express the hope that sometime before the morning is over we will have the pathology and physiology of burns integrated into the picture.

(Dr. Alexander S. Wiener, Brooklyn, N. Y.) I noticed that the water content of the tissue is given as high in one condition, and low in the other, but no figure is given for the normal.

(Dr. McGrew) We made no lengthy comparison with the normal. We did it in several dogs. We have not enough figures to give a series for normal dogs, such as we have for shocked dogs and for dogs following hemorrhage. The outstanding thing is that in practically every case the shock-tissue showed an increase in the amount of water over that contained in the hemorrhage-tissue. Whether the amount in the animal after hemorrhage is practically normal I cannot say at the moment. We can call it an increase over the hemorrhage value, or an increase over the normal.

(Dr. Theodore J. Curphey, Hempstead, N. Y.) In one of your experiments you had evidence to show that a small amount of hemorrhage in a shocked animal produced rather sharp change. Have you any evidence to show the effect of the shocking factor in an animal who is in the mid-stage of the experimental picture with hemorrhage—just the reverse of that phenomenon?

(Dr. McGrew) No, we have no experiment exactly like that, although we did combine the two to produce serious shock and serious hemorrhage in a series of dogs, and in these all I can tell you is that the blood pressure in every case fell rather dramatically and rapidly, but the physical findings and the chemical findings of the blood are those of the predominant factor; if there is more shock than hemorrhage you will get a shock-picture, although you can imagine with the combination of the two the blood pressure will fall rapidly.

it did not matter which method produced it; the essential characteristics were the same, although they varied in degree.

(Dr. Alfred Plaut, New York, N. Y.) We saw calcification in one of the pictures presented to us; what about the time factor? How long after the shock took place were these animals killed, or did they die?

(Dr. Lieber) The 2 dogs that were burned lived until the eleventh day, at which time we killed them because of the discomfort they had from the burns on the body.

(Dr. Otto Saphir, Chicago, Ill.) I should like to know if you have any data on the postmortem findings on patients who have died as a result of shock, and if so, whether the changes are similar to those experimentally produced.

(Dr. Lieber) It is very difficult to interpret changes as they occur in human beings. It is not often we have the opportunity to examine the tissues sufficiently early after death, since postmortem changes occur so rapidly, much earlier in shock than in other conditions; so I would be very hesitant in interpreting changes as they occur in human patients unless I could obtain all the material within 2 hours after death.

(Dr. Tracy B. Mallory, Boston, Mass.) I should like to ask how many of the changes you have just described are present in animals in which a clinical degree of shock can be recognized and then are sacrificed some hours before they might otherwise die. If you sacrifice animals as soon as a clinical degree of shock can be recognized, do you find all these visible lesions?

(Dr. Lieber) No; the changes as they occur early are simply congestion and edema. With severer degrees of shock and early death petechial hemorrhages are found. The degenerative changes are seen in various viscera, but focal necroses as they occur in different tissues are usually associated with a severe degree of shock over a longer period of time; by that I mean over 10 hours.

THE TREATMENT OF SHOCK BY THE INTRAVENOUS ADMINISTRATION OF NON-HEMATOGENOUS MACROMOLECULAR SUBSTANCES. W. C. Hueper and (by invitation) G. J. Martin and M. R. Thompson, New York, N. Y.

Abstract. A rational treatment of shock must counteract not only the impaired circulation, the lowered blood pressure and the increased blood viscosity brought about by the escape of plasma through the vessel walls, but must obviate also the effects of the primary and secondary endogenous factors causing an excessive capillary permeability and a defective vascular tonus. The two macromolecular agents, plasma and gum arabic, used for this purpose not only fulfill relatively imperfectly these fundamental requirements, but offer also certain difficulties in their practical use because of limited availability, complicated preparation and handling, lack of standardization, acute allergic or toxic reactions, etc. Experiments were conducted with 0.5 to 1 per cent aqueous colloidal solutions of methyl cellulose fortified by the addition of the natural detoxicants, ascorbic acid, cystein hydrochloride, calcium glucuronate and glycine. These solutions were injected intravenously into dogs subjected to shock by the application of bags filled with crushed dry ice to the shaved skin or by the subcutaneous administration of histamine in an oil-lanolin emulsion. It was found that this medication

apparently the opposite — a fall in the histamine content. I should like to ask the authors whether either of them has comments to make on that situation.

(Dr. Rose) The experiments of Dragstedt were performed on dogs, and I think the work shows very well that the amount of histamine in the plasma of the dog increases considerably. In the rabbit, on the other hand, we have shown that there is a marked decrease of the histamine content of the blood during anaphylactic shock. I should like to emphasize, however, that the results presented today were obtained from patients with shock due to burns and in rabbits during hemorrhage and shock due to trauma.

(Dr. Moon) Then this feature as seen in the rabbit is opposite to that in the dog?

(Dr. Rose) Yes.

MORPHOLOGIC CHANGES IN EXPERIMENTAL SHOCK. Marshall M. Lieber and David R. Morgan, Philadelphia, Pa.

Abstract. Shock of varying degree and intensity was produced in dogs with diverse agents. These included tissue substances introduced intraperitoneally, burns, peptone poisoning, anaphylaxis, intestinal obstruction, roentgen irradiation of the abdomen, and others. The visceral changes after death in 113 dogs in this series included generalized capillo-venous congestion, most prominent in the liver, kidneys, gastro-intestinal mucosae, lungs and serosae. Frequently petechial hemorrhages were noted in these tissues and also in the epicardium, meninges and brain. When death was delayed, the soft tissues were edematous and there were effusions into the serous cavities. Histologically, capillo-venous congestion, edema and occasionally petechial hemorrhages were marked. Parenchymatous degeneration of the viscera was the rule. Focal necroses were noted in the liver, spleen, lymph nodes and adrenal glands in a number of animals. These morphologic features are directly related to capillary injury with increased permeability resulting in circulatory deficiency and anoxia of the tissues.

It is to be emphasized that the various tissues present a variety of changes which are dependent on several factors including the nature of the shock-producing agent, the intensity or quantity of that agent and the period of time over which it acts before death is produced. The severity of the pathologic changes does not always parallel the degree of shock produced. Under identical conditions of experimentation, the degree and distribution of the changes may vary in different animals of the same species.

Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I should like to ask whether the lesions produced are attributable to shock or the substances used to produce the shock. The focal necrosis in the liver and spleen, for example, would be a little more easily explained as the result of these toxic proteins that are liberated by the necrotic tissue.

(Dr. Lieber) We interpret these as probably due to the effect of these agents on the capillary endothelium, resulting in circulatory deficiency with anoxemia and anoxia of the tissues.

(Dr. Bell) Do these occur in any kind of shock?

(Dr. Lieber) We used all these various methods of producing shock, and

materials needed to correct the hypoproteinemia as well as nitrogen for other body protein requirements. The dried digest is a golden yellow, granular material containing 12.5 per cent nitrogen. In a 5 per cent solution, sterilized by Seitz filtration, it is well tolerated when given either intravenously or subcutaneously.

Discussion

(Dr. Stuart Mudd, Philadelphia, Pa.) I think the utility of this procedure of feeding plasma proteins over a period of weeks is perfectly clear. I am wondering if Dr. Whipple has had any experience with it in such emergencies as burns, in which plasma may be given intravenously as an emergency measure for a week. Might it be possible to give plasma intravenously for a day or so, and to give such digests for the remainder of the emergency?

(Dr. Whipple) That is the hope we have, but a great deal of trial under clinical conditions will be necessary. Certainly this digest should be of value. It can do no harm, as we see it. How well it will be utilized, and how much plasma might be given under these abnormal conditions of shock I do not know.

(Dr. Alexander S. Wiener, Brooklyn, N. Y.) This work seems to open up many brilliant possibilities. One question I should like to ask is whether Dr. Whipple has any idea as to where the serum albumin is produced.

(Dr. Whipple) That is a nice question. My belief is that the albumin is produced in the liver, but others have different ideas. The evidence, I think, is accumulating that a good deal of the albumin, perhaps all of it, is produced in the liver. Certainly there are certain globulins (fibrinogen) produced in the liver, and the evidence indicates only in the liver. Other globulins appear to be produced outside the liver. Further than that one is on very debatable ground.

THE VASCULAR AND CELLULAR DYNAMICS OF SHOCK. Virgil H. Moon,*
Philadelphia, Pa.

Abstract. Shock has been under intensive investigation for years, resulting in much information but not in complete agreement of interpretation. Confusion has resulted chiefly from four major causes.

1. Incomplete knowledge of the functions and reactions of capillaries delayed clarification of the problem. Endothelium is highly susceptible to the effects of various agents and conditions. These include bacterial substances, foreign proteins and split-products, extracts of normal tissues, histamine, bile, venoms, chemicals, poisons, metabolic products and even moderate lack of oxygen. Any type of injury to endothelium increases its permeability to plasma colloids. Leakage of plasma from the blood produces hemoconcentration, lowers the total blood volume and leads to a disparity between it and the volume capacity of the vascular bed. This disparity, if uncompensated, manifests itself in the characteristic signs of shock. The syndrome is accompanied by distinctive physiologic disturbances and pathologic features which are related directly to endothelial damage. Abnormal permeability of endothelium disturbs seriously the mechanism of water balance. Normal movement of fluid between the blood and the tissues depends upon several factors, including capillary blood pressure, osmotic pressure, concentration of electrolytes and others. But the action of these forces,

* By invitation of the Council.

is efficacious and relatively safe, as it reduced quickly the hemoconcentration of the shocked animals in many instances; it prevented the development of the prognostically unfavorable marked leukopenia of cold shock; it seemed to have exerted a definitely life-saving effect in 3 dogs with histamine shock which exhibited leukocytoses between 55,000 and 63,000 cells, being in the lethal range, according to Moon. Whereas the osmotically active, macromolecular methyl cellulose solution cannot be considered as an adequate substitute for plasma, it possesses certain properties recommending it for further study as a more suitable non-hematogenous blood substitute than gum arabic. Methyl cellulose is produced in this country; it is easily sterilized by boiling and is refractory to bacterial infections of the ordinary type; much smaller amounts of methyl cellulose are necessary for producing a solution having the same viscosity as plasma than are required with gum arabic; methyl cellulose is more adaptable as it comes in several grades of viscosity reflecting differences in molecular size; it is not more hazardous as to secondary storage phenomena than gum arabic. The detoxicants added to the methyl cellulose solution apparently accentuated and supplemented the beneficial effect produced by the macromolecular agent.

Discussion

(Dr. Bram Rose, Montreal, Canada) I should like to ask if Dr. Hueper controlled any of these experiments by the injection of an equivalent amount of saline. You can prevent a certain amount of shock by the injection of normal physiological saline.

(Dr. Hueper) We have not injected normal saline because we do not think it is the solution which should be used in shock treatment. We need a colloidal macromolecular agent over a long period to sustain the plasma volume, which cannot be done by a medium like saline solution, or dextrose.

(Dr. Stuart Mudd, Philadelphia, Pa.) Have you made any attempt to apply this clinically?

(Dr. Hueper) No; our work has been only experimental.

(Dr. Greenblatt, New York, N. Y.) What is the specific rôle of ascorbic acid in these experiments?

(Dr. Hueper) It is well known that various detoxicants act rather specifically on certain toxic agents; ascorbic acid, for instance, detoxicates benzol and its derivatives; that is well known from a large number of experiments.

(Dr. Theodore J. Curphey, Hempstead, N. Y.) Have you had any toxic reactions in your animals?

(Dr. Hueper) No.

(Dr. Eric Ponder, Mineola, N. Y.) What is the osmotic pressure of the solution of methyl cellulose compared to that of plasma protein?

(Dr. Hueper) Practically isotonic.

SHOCK: PLASMA PROTEIN BUILDING IN EMERGENCIES AS INFLUENCED BY INTRAVENOUS DIGESTS. G. H. Whipple, Rochester, N. Y.

Abstract. By established technic for the measurement of plasma protein production in hypoproteinemic dogs, we have determined that an enzymatic (papain) digest of commercial casein given parenternally is as effective in plasma protein production as whole liver by mouth. This digest provides

It seems remarkable that these contrasts have escaped the attention of many who have discussed hemorrhage as related to shock. Hemorrhage, when present, is a highly important contributory factor, but distinctions between the accompanying physiologic disturbances invalidate the assumption that shock and the effects of hemorrhage are identical.

3. Factors of error inherent in experimental methods constitute a third major source for confusion. A method commonly used is to narcotize an animal deeply, then to produce extensive trauma to the tissues, using a decline in blood pressure as the indicator of shock. This method is open to serious objections. It is well known that the blood pressure may fall during shock or after hemorrhages or from deep narcosis. In some reports the workers showed conclusively that they were dealing almost entirely with hemorrhages; others recorded the marked depressor effects of the anesthetic. Under such conditions the result may be due to the narcotic, to absorption from traumatized tissues, to the associated hemorrhages or in part to each. This combination of indeterminate factors has led to undependable conclusions and to confusion.

Methods were devised for testing the effects of absorption uncomplicated by anesthesia and by hemorrhage. The implantation of tissue pulp into the peritoneal cavities of normal dogs produced regularly a circulatory deficiency having the characteristic features of the shock syndrome. This was accompanied by progressive hemoconcentration and by chemical alterations in the blood such as occur in traumatic or surgical shock and after extensive burns. Watery extracts of normal tissues produced similar effects when injected. These results confirm the interpretation that the absorption of products from damaged tissues is a factor in producing circulatory failure.

4. Finally, the belief that shock is purely a physiologic disturbance, unaccompanied by significant morphologic features, hindered the clarification of the problem. A characteristic pattern of morphologic visceral changes develops both in clinical and in experimental shock (*vide supra*). Such findings are indicative of endothelial damage and support the interpretation that various agents, including products absorbed from damaged tissues, exert deleterious effects upon capillary walls and thereby initiate a group of physiologic disorders leading to circulatory deficiency and to lack of oxygen in the tissues. This factor of itself causes endothelial permeability and thereby introduces a self-perpetuating feature which gives the mechanism the quality of a vicious circle shown diagrammatically on the following page.

Recent Theories

The Alarm Reaction. Selye described a syndrome resulting from severe damage but independent of the nature of the damaging agent. The term "alarm reaction" was applied to the sum total of the adaptive or defensive reactions against the effects of the damaging agent. The manifestations of shock were interpreted as due to inadequacy of the defensive reactions. When these are adequate, a second phase, "countershock," develops and is characterized by a reversal of the clinical signs of shock. This conception omits essential mechanisms from consideration. Shock, interpreted as inadequacy of physiologic defenses, presents an uncompleted picture. One still inquires by what mechanism is the circulation disturbed and why are the metabolism, renal function and chemical concentrations altered.

in maintaining physiologic relations between intravascular and extravascular fluids, is absolutely conditioned upon the presence of a normal semi-permeable membrane—the endothelium—between them. Circulatory deficiency of this type is accompanied by vomiting, diarrhea, edema, hemoconcentration and by inability to absorb fluid from the tissues. These features indicate disturbances of fluid balance.

Abnormal *cellular* permeability also is an important feature of pathologic physiology. The outer surface of each living animal cell functions like a semi-permeable membrane. Hence each cell behaves as an osmotic unit. Living protoplasm maintains chemical concentrations differing markedly from those of the external fluids, but this property is reduced by any kind of cellular injury and is lost entirely as the cell dies. This allows the differences in concentration to be equalized and accounts for the high potassium content, hypochloremia and other chemical alterations of the blood during shock. This type of circulatory failure may develop after extensive traumatic injury, surgical procedures and burns. It occurs also incident to abdominal emergencies, intoxications, infections of unusual severity, serum disease and in acute poisoning of various kinds. When it follows trauma or surgical procedures, it usually is complicated by hemorrhage and by other factors, but it can be produced in uncomplicated form by various agents which injure endothelium. Adequate understanding of capillary reactions and function has removed one major obstacle to a comprehension of shock.

2. A second cause for confusion has been the failure to distinguish between shock and the effects of hemorrhage. Any type of circulatory deficiency activates the sympatho-adrenal system. This stimulates the myocardium, increases the strength and rapidity of the pulse, excites peripheral vasoconstriction, thereby causing peripheral ischemia, declining temperature and loss of tissue turgor. It causes an increase in the blood sugar, dilatation of the pupils and perspiration. Low basal metabolism, increased respiratory rate, thirst and declining blood pressure accompany both shock and hemorrhage. Hence several of the clinical signs are identical, but other accompanying features are opposite in character.

In shock the capillary endothelium becomes permeable, the tissue fluid and flow of lymph are increased, fluid balance is disturbed, ability to absorb fluid from the tissues is impaired, vomiting and diarrhea are frequent and infusions of fluids or transfusions of blood often are ineffective in treatment. None of these features result from simple hemorrhages.

In shock the blood becomes concentrated, the nonprotein nitrogen, potassium, calcium and magnesium contents are markedly increased, the sodium, chlorides and carbonates are decreased, the coagulation time is lengthened and the sedimentation rate is retarded. None of these changes are produced by hemorrhages.

During shock the urine is decreased in volume and contains albumin, erythrocytes, casts, bile and other abnormal substances. No characteristic urologic changes result from hemorrhages.

The necropsy findings after death from shock include edema, serous effusions, capillo-venous congestion, stasis and petechiae in the viscera, atony of the gastro-intestinal tract, focal necroses and acute degeneration of parenchymatous organs. None of these morphologic features result from the effects of hemorrhage.

Sympatho-adrenal Hyperactivity. The excessive effects of epinephrine will produce the complete syndrome of shock. This may result from tissue anoxia produced by maximal arterial constriction. Freeman has proposed that shock following trauma, surgery or burns similarly may result from hyperactivity of the sympatho-adrenal system evoked by pain, emotions or injury to the tissues. The theory is questionable in several particulars. It has not been shown that the animal's own glands can produce enough epinephrine to cause shock within the time limits of the experiment. Neither excessive pain nor prolonged stimulation of nerve trunks will cause shock, nor will cutting the cord or severing all nerve paths from the traumatized area prevent its development. Others have reported that animals subjected to adrenalectomy or to sympathectomy are as susceptible to shock from trauma or other agents as are normal animals. Activity of the sympatho-adrenal system is maximal during physical combat, yet this does not cause shock independent of injuries.

Roentgen irradiation of the abdomen causes delayed necrosis of intestinal mucosa accompanied by delayed shock. This treatment is painless and provides no cause for delayed sympatho-adrenal hyperactivity.

Evidence supporting this theory was derived from a comparison of the effects of hemorrhages in sympathectomized and in normal dogs. Since shock and the effects of hemorrhage are not identical (*vide supra*) these experiments do not bear directly upon the problem. If the author could show that sympathectomized animals are resistant to the effects of several shock-producing agents, he would increase greatly the weight of his evidence.

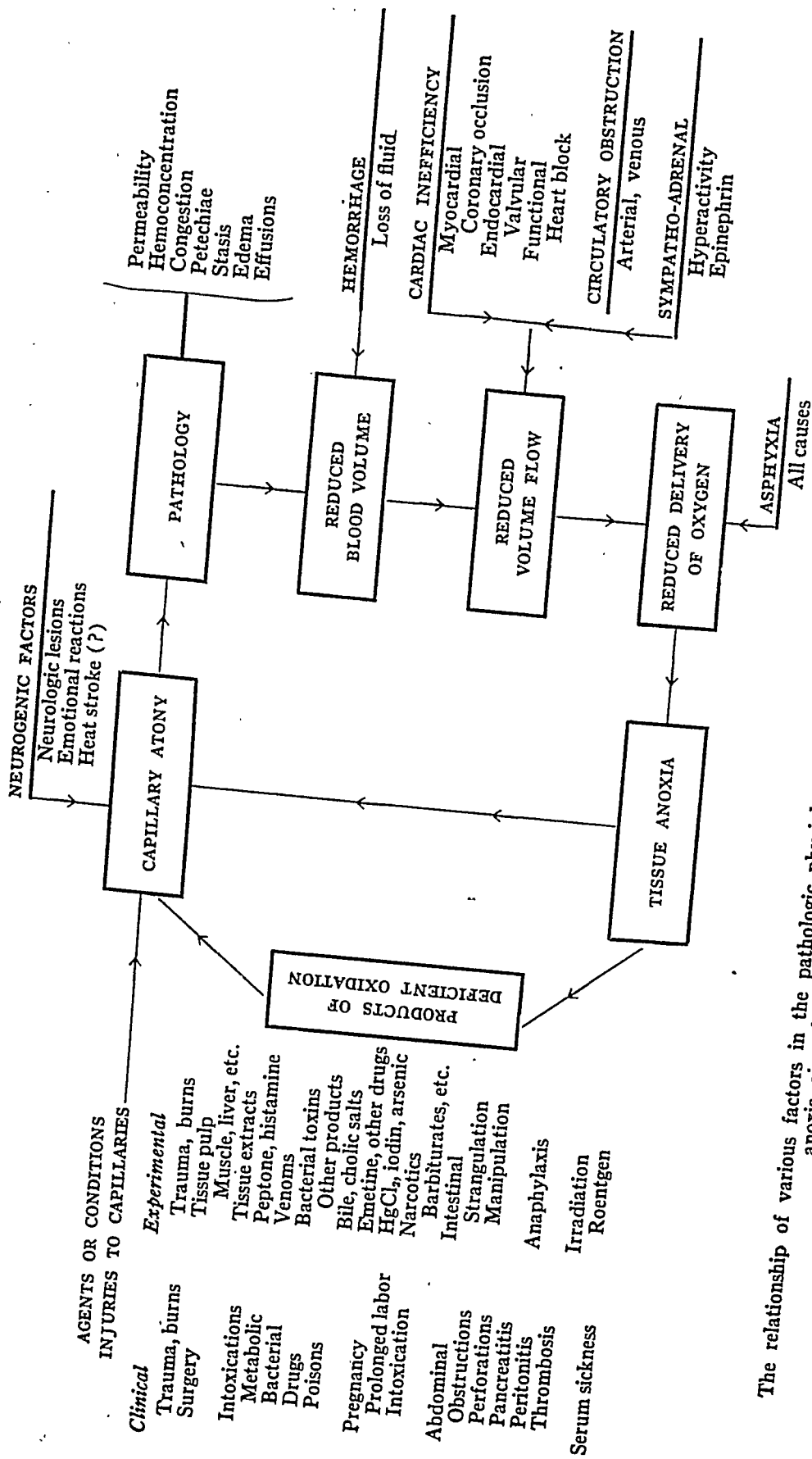
THE RÔLE OF POTASSIUM IN THE SURVIVAL TIME AFTER BILATERAL NEPHRECTOMY. S. Durlacher and D. Darrow (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. The survival time after bilateral nephrectomy varies considerably, but in general far exceeds that after renal artery ligation. This has been shown to be related to the rise in the potassium of the blood. When animals are fed on a low potassium diet until the potassium content of their tissues and serum has been reduced, their survival time following bilateral nephrectomy is significantly increased over control animals fed a normal diet. In the latter the nonprotein nitrogen of the blood rises rapidly and the serum potassium is found to be at fatal levels at the time of death, whereas in the former the animals die after a prolonged period, with markedly elevated nonprotein nitrogen and serum potassium levels that are not in the fatal range.

DISUSE ATROPHY OF RENAL ARTERIES. E. T. Bell, Minneapolis, Minn.

Abstract. In chronic glomerulonephritis atrophy of the renal cortex may result from extensive obliteration of the glomeruli, and in chronic pyelonephritis destruction of the tubules in the medulla or corticomedullary junction may bring about atrophy of areas of the cortex. In neither of these diseases is the cortical atrophy due to vascular obstruction, but the arteries supplying the cortical scars may show appearances which are easily confused with primary arterial disease.

This alteration in the arteries is due to the circumstance that their function is reduced to a minimum, and the change may appropriately be called



The relationship of various factors in the pathologic physiology of shock. The reciprocal effects of capillary atony and tissue anoxia give this mechanism the self-perpetuating quality of a vicious circle.

cortical extract have been employed in assaying the strengths of different lots of extract. The normal sodium content of the blood plasma in adrenal insufficiency is usually decreased about 15 per cent and chloride content somewhat less, both phenomena being due to excretion of these substances in the urine. There is a simultaneous increase of potassium and magnesium. There is a close similarity of the blood picture of adrenal insufficiency and of chronic nephritis. With the loss of sodium chloride, there is in both conditions an increase in the potassium and magnesium serum content. Equally important are changes noted in the kidney tubules in adrenalectomized animals. These changes noted by many observers were first described by Marshall. The relation between activity of the adrenal and blood pressure is significant. The low blood pressure in Addison's disease and adrenalectomized animals, the increased blood pressure in hyperactivities and following the injection of synthetic preparations, and the reduction in blood pressure in the Goldblatt hypertensive cases following adrenalectomy have been frequently noted. Smith, calculating from plasma clearance (diodrast) and the filtration of water from the blood in the glomeruli (clearance of inulin), demonstrated in early renal hypertension an early loss of tubular function. The increased glomerular pressure is dependent on constriction of the efferent arterioles, which increases the filtration pressure and the flow of blood through the kidney. Following this vasoconstriction and ischemia, afferent arteriolar changes occur. That the vascular changes in the afferent vessels are secondary to hypertension and not primary appears likely for many reasons.

While the conservation of salt and water is due essentially to its reabsorption in the kidney, this is not the only organ where such reabsorption occurs. There is reabsorption of cerebrospinal fluid by the blood sinuses, of intraocular fluid, of salt and water in the gallbladder and of salt and water in the large bowel. In all these organs responsible for reabsorption we have hypertensive vascular lesions. The pancreas is intimately related to salt metabolism and with adrenalectomies performed years ago a constant pancreatic lesion was noted. We believe that the activities of the adrenal and pituitary are responsible for the reabsorptive processes everywhere. While comparisons are admittedly difficult and hazardous, the similar changes in vessels, for instance, in the glomerulus and choroid plexus and the marked disturbances in a main function common to both organs (the reabsorption of water and salt) make the suggestion of a similar etiology of hypertension in these organs seem natural.

As expressed by F. P. Parker, the preservation of the normal chloride is essential to the maintenance of the proper physical equilibrium of the various electrolytes of the tissues and body fluids which influence the distribution of water between the circulatory blood and the tissues. The reabsorption of water and salt to permit the excretion of nitrogenous material is the combined function of normal kidney tubules and the adrenal gland. Failure of either tubules or adrenal cortex will result in loss of salt, accumulation of nitrogenous products and a reconstitution of the various electrolytes in the blood. Addison's disease follows destructive lesions or atrophy of the adrenal gland and a failing hypertensive kidney is characterized by decompensation and hyperplasia of the kidney tubules. Failure of the kidney either in essential or secondary hypertension is dependent on tubular decompensation. With tubular failure, and the loss of sodium

"disuse atrophy." The lumen of the affected vessel is reduced in size and its walls appear relatively thick in proportion to the size of its lumen. The media has a glassy, semihyaline appearance. The medial change is due to medial fibrosis—a replacement of smooth muscle by collagenous fibers. In the afferent arterioles this is the only alteration, but in the small arteries one sees, in addition to medial fibrosis, a marked folding of the internal elastic lamina corresponding to the decreased size of the lumen. In the medium-sized arteries there may be an elastic intimal thickening, which is independent of hypertension since it occurs in chronic pyelonephritis without hypertension. Uncomplicated disuse atrophy of small arteries and arterioles differs from primary vascular disease in the absence of intimal changes.

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) As Dr. Bell has pointed out, it is very desirable indeed to distinguish between uterine and renal artery changes. The fibrosis of the media of small arteries and arterioles, emphasized by Dr. Bell, is important. I do not think the evidence is at all conclusive that it is a manifestation of disuse atrophy. By narrowing the ureters, medial necrosis followed by fibrosis may be produced. Different viewpoints exist concerning the fate of the nephron when the glomerulus or a part of the tubule is destroyed. Tubules may persist, as has been shown by Dr. Oliver and his associates, even in infarcts. This is important evidence.

(Dr. Bell) The only thing I can say is that in this form of disuse this is the type of change we see. Whether it can be produced in some other way or not I do not know. Probably it might result from some other cause. As to the nephron not disappearing when a part of it is destroyed, I am aware of Dr. Oliver's work, but I have never been able to find, in tracing serial sections, any such thing as that — if a glomerulus is hyaline you invariably find it associated with an atrophic tubule.

UREMIA AND PERICARDITIS. FINDINGS AS THEY RELATE TO HYPERTENSION.

Leon K. Baldauf and (by invitation) Robert E. Ingersoll, Cambridge, Mass.

Abstract. The primary and fundamental function of the kidney is the regulation of the inorganic composition of the plasma rather than the obvious excretion of nitrogenous waste products. In uremia with pericarditis there is always marked nitrogenous retention in the blood, a high blood pressure, a persistent acidosis and a tendency to hemorrhage. Blue and co-workers maintained that the azotemia was due to a loss of salt. Causes for this deficiency include kidney failure and cardiac failure with accumulation of fluid in the cavities, use of diuretics, particularly acid salts and mercurials, vomiting, and insufficient chlorides in the diet. This marked nonprotein nitrogen retention and finally dehydration with further concentration of salts in the serous cavities account for the granular and crystalline deposit on the pericardial surface which we consider the primary and essential pericardial lesion.

The adrenal cortex is frequently spoken of as supplying the water-salt hormone. In Addison's disease and in adrenalectomized animals the amount of nonprotein nitrogen is greatly increased. Indeed, in many cases, the rise of nonprotein nitrogen and the corresponding fall after restoration with

failure, there is no doubt, even at low magnifications, of the presence of vascular disease. The striking feature in this series of biopsies was that at first glance many of them appeared normal, but when searched for, vascular disease was evident in every biopsy. The vascular lesions found were classified as intimal hyalinization, medial hypertrophy and degeneration and endothelial hyperplasia. No necrotizing arteriolitis was observed—further confirmation of the theory that this lesion is a terminal change. Most of the cases showed combinations of these types, but in a few one type was predominant. No specific type of disease was limited to any one size of vessel, although many of the diseased small arterioles showed only medial hypertrophy. There were 4 cases, 2 with a definite history of infection, in which the biopsies showed focal scarring consistent with pyelonephritis according to the criteria of Parker and Weiss. The vascular disease, however, was not limited to the scars, although it was much more severe in those areas. Independent efforts to grade the severity of the process by clinician and pathologist showed a fair degree of correlation, although in general there was a tendency for the clinical grades to be more severe. Valid criticism of these gradings might be made on the basis that the biopsy was too small to be representative of the whole kidney. We admit this possibility, but since most of the kidneys appeared uniform at operation and were the same on both sides, we feel that each biopsy was a fairly good sample.

From these findings it is quite obvious that there is evident renal vascular disease in hypertensive patients long before there is any clinical suggestion of renal failure or any serious morbidity. These findings, therefore, do lend some support to the theory that the renal disease precedes the hypertension. On the other hand, there are perhaps some clinical results that are pertinent to this problem. Before this small group of patients was studied, 30 hypertensive patients essentially similar in all respects to the present group, were subjected to the same kind of sympathectomy. This operation consists of a bilateral total splanchnic denervation, the great splanchnic nerves being removed from the semilunar ganglion to approximately the midthoracic level and the sympathetic trunk resected from D-9 to L-1 or L-2 inclusive. These 30 patients have been followed for periods of 3 months to 2½ years with the following results: Sixty per cent of them now have normal or near normal blood pressures, most of them responding immediately following the second stage of the operation. Although no renal biopsies were taken in this series, it is reasonable to assume that had they been made, renal pathology similar to that found in the recent biopsied series would have been observed. It is much too early to make any statement on the follow-up of the biopsied cases, but it is also reasonable to infer from the experience of the previous larger series that, since 14 out of the 16 biopsied patients had an immediate response postoperatively, most of them will be markedly benefited by the operation in spite of their definite renal disease. This is merely a preliminary report indicating anatomic findings only and no attempt is made at this time to fit it into any theory as to the cause of hypertension, or even to suggest that the vascular disease may be reversible. Further data such as tests for renal blood flow preoperatively and postoperatively, pressor substance determinations from the peripheral and renal veins which are now being collected from these patients, and the condition of their kidneys in years to come may shed further light on the subject.

chloride, hyperactivity of the adrenal follows in the attempt to establish salt equilibrium. Hyperactivity of the adrenal cortex as a compensatory effort to overcome the loss of salt from decompensated tubules may thus give rise to secondary hypertension, while the hyperactivity of the gland itself with failure of the reabsorptive processes may lead to what is generally known as essential hypertension.

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) In Dr. Ingersoll's comprehensive review of medicine, I did not understand what type of pericarditis he had in mind—whether it was fibrinous or another variety.

(Dr. Baldauf) Many of the cases of pericarditis are not fibrinous in nature. Covering the pericardial surfaces is a fine granular and crystalline material. We believe this to be the essential and primary lesion. The fibrinous lesion we feel is secondary to this lesion: in some instances due to a terminal infection; in other instances to a chemical irritation with high blood urea and a tendency to hemorrhage.

RENAL BIOPSIES FROM HYPERTENSIVE PATIENTS. Benjamin Castleman and (by invitation) Reginald H. Smithwick and Robert S. Palmer, Boston, Mass.

Abstract. During the past 4 months we have had the unique opportunity of studying renal biopsies removed from 16 hypertensive patients who were being subjected to sympathectomies. The selection of patients for sympathectomy is very difficult and one of the purposes of this study was to learn how much renal damage, if any, there actually was in patients with relatively early hypertension who had no clinically demonstrable renal impairment, and conversely, how extensive the renal damage was in patients with renal insufficiency. During the course of the operation, usually following the nerve resections, a wedge-shaped biopsy approximately 6 to 7 mm. wide and about 5 mm. deep was taken. When biopsies were taken from both kidneys, the gross and histologic appearances on each side were essentially similar.

The patients included in this series were young individuals, only 2 being in their forties and the others ranging from 20 to 38 years of age. Their blood pressures were over 200 systolic and the symptoms of hypertension had been present on the average for 2 years, although in 3 patients the history went back for only a few months and in 3 others for 10 to 12 years. All showed some retinal change. Eleven out of the 16 had normal renal function as measured by the PSP test. Except for 1 patient who should not have been selected for operation, none of them approached the stage in their disease where demise seemed imminent.

The kidneys were fully exposed, found to be normal in size and only rarely were the capsules unduly adherent to the cortex. Occasional tiny scars were visible in about half the cases but most of the parenchyma was smooth and looked normal to both surgeon and pathologist. The biopsies were usually taken through normal-appearing cortex and in a few cases included a portion of a scar.

When one examines microscopically the kidney removed at autopsy from the average hypertensive patient, including those not dying from renal

THE MORPHOLOGICAL ASPECT OF THE GOORMAGHTIGH CELLS (JUXTAGLOMERULAR APPARATUS) IN THE NORMAL AND DISEASED HUMAN KIDNEY. William Kaufmann (by invitation), Albany, N. Y.

Abstract. Following a previous preliminary report, approximately 400 kidneys removed surgically and at autopsy were examined for the presence of the juxtaglomerular apparatus or "Goormaghtigh cells," as we shall call the cells in recognition of the man who first described them. Various fixing and staining technics were used and Goormaghtigh cells were found regularly at the vascular pole of many but not all glomeruli, along the vas afferens and even along the vas efferens and the interlobular artery in kidneys of patients ranging in age from 3 months to 75 years.

Special attention was given to cases of progressive arteriolar nephrosclerosis, malignant nephrosclerosis and chronic pyelonephritis with secondary vascular changes, all with clinical evidence of hypertension. In these cases hypertrophy and hyperplasia of Goormaghtigh cells were uniformly observed, but the finding of increased acidophilic or basophilic cytoplasmic granules, as seen in experimental renal ischemia in dogs by Goormaghtigh, and in rabbits by Dunihue and Candon, could not be substantiated. The preservation of Goormaghtigh cells in cases of advanced sclerosis of the arterioles appears to support Goormaghtigh's experiments, in which feeding of massive doses of vitamin D₂ or calciferol to dogs produced arteriolar necrosis in the renal arterioles, but left the cells of the juxtaglomerular apparatus intact for a long time. In the absence of definite cytoplasmic granules, the hypertrophy and hyperplasia of Goormaghtigh cells in our patients with hypertension constitute the only morphological basis for their possible relationship to the formation of a renal pressor substance.

Discussion

(Dr. Francis Bayless, Cleveland, O.) I should like to congratulate Dr. Kaufmann on his very beautiful kodachromes. In Cleveland we have had opportunity to examine the kidneys of a number of Dr. Goldblatt's animals—monkey, dog and goat—and some material from hypertensive human beings. In the animal series there were normal kidneys, kidneys that were ischemic, and kidneys contralateral to an ischemic kidney. The animal material we studied was received as unknown; the tissue was fixed in Bouin's, in Zenker-formol, or in formalin, and stained by various methods. We can confirm the observations of Dr. Goormaghtigh, Dr. Kaufmann, and others, and the chief point of interest now is the interpretation of the findings. In some instances the juxtaglomerular apparatus appears to be larger in renal ischemia and in some instances larger in hypertension. The presence of this apparatus in the normal kidney is quite easy to establish by anyone who cares to look. The most troublesome thing is the inconstant finding of granules in the cytoplasm of the *Polkissen* cells. They vary in number, depending on the species of the animal. In human beings they seem to be very hard to find, although they are encountered. In some animals they are more common, and seem to have a relationship to the hypertensive state or to renal ischemia. The further question to be decided concerns the possible relationship between enlargement of the juxtaglomerular apparatus and the presence or absence of so-called Ludwig's vessels in the kidney. We are now trying to establish whether that vessel is normally present in the kidney,

Discussion

(Dr. M. C. Winternitz, New Haven, Conn.) I think this is an important and interesting contribution. It has been difficult to understand just why sympathectomy should be effective clinically. The demonstration of the changes in the renal artery is interesting. Dr. Castleman has made a conservative and clear presentation of the facts.

(Dr. Joseph E. Smadel, New York, N. Y.) Would Dr. Castleman care to discuss early changes observed in the glomerular tufts of his sections?

(Dr. Castleman) These biopsies were done during the last few months and we expect to go further in the examination of these kidneys for early changes. In most of the cases, however, the glomeruli appeared perfectly normal. No diagnostic early changes were noted.

(Dr. Irving Graef, New York, N. Y.) Pertinent to this report is a case I had the opportunity of studying last year and upon which I reported at the Pittsburgh meeting. It has since had an interesting follow-up. In the study which I was making, evaluating Goormaghtigh's claims of the so-called juxtaglomerular apparatus, a kidney was obtained by surgery from a female 30 years of age who was pregnant and who had had hematuria for a period of 5 years. Because her obstetricians were loathe to carry her through pregnancy with the hematuria, fearing some obscure complication, exploration was advised. The urologist exposed a grossly normal kidney and on splitting the pelvis revealed the presence of blood, but no obvious changes as the source of it. Small blocks and the rest of the kidney were fixed *in toto* at once. Histological sections studied serially revealed the presence of massive medial hypertrophy affecting the entire arteriolar tree and extending backward for a considerable distance into the interlobular arteries. The patient at the time had no hypertension. She had been hospitalized on five previous occasions and on none of them had shown any hypertension. She had no impairment of renal function, except the hematuria from the left kidney. We could not account for the vascular change. It corresponded with the changes described by Dr. Goormaghtigh in some of his cases of hypertension, but this patient did not have hypertension.

In May, after I had reported this case at the Pittsburgh meeting, the patient suddenly developed eclampsia, with hypertension, and was delivered of a living child. Subsequently her hypertension disappeared and today she is normal, a little more than a year after the nephrectomy. I cite this case again at this point simply to indicate that the renal arteries may behave in a remarkable manner, exhibiting marked medial hypertrophy, without hypertension. Whether this patient will develop hypertension I do not know, but I feel we have a great deal yet to learn about the behavior of medial muscle under various physiological and abnormal conditions. This contribution of Dr. Castleman's is consistent with everything we know about established hypertension in which we are certain that a number of mechanisms are operating to affect the vasculature of the body, not only the media, but the intima as well. As Dr. Winternitz and his associates have shown, we can dissociate many of the effects of this curious renal disease, some of them leading to necrosis of the vessel walls, some to changes in the capillaries, and some to changes in the smooth muscle. I believe it would be wrong for us to keep on simplifying our concept of the relationship of hypertension to arteriolar disease and ascribe every instance of hypertension to similar changes in the vessels, or all changes in the vessels to hypertension.

it is very easy to discover granules in the Goormaghtigh cells and, as Dr. Bayless mentioned, anybody who cares to look for them will find them. The difference between the myofibrils of the smooth muscle cells of the arteriolar media and the granules of the Goormaghtigh cells is readily visible in most instances. Also the granules are extremely large in comparison to the fine mitochondria of the tubular epithelial cells. They have a more rounded appearance and look somewhat like the granules of an eosinophilic leukocyte.

From the studies of our human material it is not possible at the present time to draw any definite conclusion as to the possible function of these cells. Two possibilities have to be considered at present: one, that the Goormaghtigh cells are functional units, responsible for the opening and closing of the preglomerular arterioles by a swelling and relaxation mechanism, possibly thus regulating the glomerular bloodflow; or second, that they are endocrine cells, as Goormaghtigh pointed out in his recent paper, which might be responsible for the production of a renal pressor substance. Investigations in this direction are being carried out presently to clarify these two theories.

"ANTI-RENIN" AND OTHER EVIDENCES OF TOLERANCE TO THE VARIOUS SUBSTANCES CONTAINED IN EXTRACTS OF KIDNEY. E. Mylon and R. Katzenstein (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. Animals injected repeatedly and over a long period with extracts of kidney always show the same vasopressor reaction (renin effect). This may be masked by a more prompt vasodilatation or a protein shock reaction, as can be proven by removal of the vasodilator substance from the extract or suppression of the shock reaction by desensitization. Some evidence for increased tolerance against the necrotizing effects that follow ischemia of the kidney is demonstrable.

FUNCTIONAL STRUCTURES IN RENAL TUMORS. Walter Schiller, Chicago, Ill.

Abstract. Most investigators have attempted to solve the much debated problem of the origin of the so-called Grawitz tumor by identifying the cellular elements of this tumor as either renal or adrenal in morphological character. Attempts to prove either the renal or adrenal origin of this tumor have both been somewhat successful on the basis of this criterion, and consequently no final decision could be reached. However, the presence of functional structures in these tumors similar to those found in the kidney during physiological activity or under pathological conditions is of far greater importance than mere morphological identity or similarity. Structures of this type are demonstrated and discussed in this paper. The storage of protein has been observed in the renal tubules, either as hyaline droplets in nephrosis, or as blood pigment in hemoglobinuria. The same change can be found in benign and in malignant renal tumors (hypernephromas). This tends to prove the renal character of this tumor tissue. On the other hand, however, these findings may help to determine whether in pathological kidneys the storage of protein is due to secretion, absorption or degeneration. Since the histological arrangement in these tumors is such as to rule out secretion or degeneration as the possible etiology of renal protein storage, absorption must be looked upon as a probable etiology of this pro-

and if so, if it becomes altered in disease. Our method combines the use of fixation and staining of one kidney with arterial injection of the opposite kidney with neoprene (a colloidal synthetic rubber mass), and then comparison to correlate gross and microscopic details.

(Dr. Irving Graef, New York, N. Y.) It is very pleasant indeed to see Dr. Kaufmann's material. He began some of his studies on this apparatus in this laboratory, and my own initial interest in the juxtaglomerular apparatus began from seeing some of his random preparations made in animals. My own experience in human material has given me great misgivings concerning the validity of observations based on some postmortem material, because fixation makes a great difference in the appearance of medial cells. Even an hour's lapse of time in a body which has been obtained at a temperature of 98.6° F. may make a difference in the appearance of the medial cells, and I have seen postmortem artefacts of all sorts which have troubled me greatly in trying to determine whether the cell arrangements were the results of fixation, or postmortem change, or both. In the few normal human cases obtained promptly and fixed adequately I have seen cells quite like those Dr. Kaufmann has demonstrated, but their random distribution and the tendency to find these cells near the outermost glomeruli with greater frequency than in the inner ones has puzzled me very much. I have concentrated rather on dogs; and I think Dr. Bayless and I have been looking at the same material, because Dr. Goldblatt was kind enough to send me biopsies of unknowns from his dog material too. I can substantiate all of Dr. Bayless's remarks with reference to the dog, and make one addition. The granular cells which are not visible in man, except perhaps once in a hundred cases, in the dog increase in ischemia. Where we are dealing with 2 kidneys, 1 normal and 1 ischemic, if the normal is exposed to hypertension we may find no more than the normal distribution of these granular cells, whereas in the contralateral ischemic kidney they are easy to find, and are obviously increased in number. Again they appear in the preglomerular portion with greater frequency, but only in the cortical glomeruli. Why this is so I do not know. It was observed by Zimmermann in 1933 in many animals, and has been confirmed by Goormaghtigh, that the outer glomeruli appear to possess an architecture which is different from the deeper glomeruli. Embryologically they are different and that may have something to do with it. But it also suggests that renal function varies with individual nephrons. It may be that these changes are purely the result of ischemia in the terminal glomeruli.

So far as the clear cells are concerned, I wonder whether Dr. Kaufmann and Dr. Bayless have any idea about the relationship of that clarity to tonus. Is it not possible that the clear cells are relaxed cells which are fixed in a state of relaxation, while the cells showing myofibrils and granular structures may be contracted cells fixed in that state?

(Dr. Kaufmann) There is nothing that I can add to Dr. Bayless's remarks. As Dr. Graef pointed out and I mentioned in my paper, it is extremely difficult to see any granules in the Goormaghtigh cells of the human kidney. Only very recently, and that means in the past few days, using different fixing fluids and different staining methods, we think we have been able for the first time to see cytoplasmic granules more clearly than before. That is all I can say on this subject at the present time.

However, in the experimental animal, such as the mouse and the cat,

contained droplets of mucin and tended to lose their polarity with relation to one another, presenting at this early stage the atypical features characteristic of the cells of a fully developed carcinoma. Occurring independently of, or in conjunction with, these changes there was the development of atypical hyperplastic changes more superficially in the mucosa. The earliest departure from normal noted in this situation were solitary small areas composed of a few irregular acini. These were lined by hyperchromatic cells which were smaller than the adjacent normal cells, the nucleus almost completely filling the cell. The stroma between the glands became more compact resulting in shrinkage and depression of the surface. The innermost layer of the deep musculature immediately beneath these hyperplastic glandular areas became thickened due to proliferation of smooth muscle cells. These newly proliferated muscle cells grew upward to meet the infiltrating glands which were progressing downward. This led to disappearance of the submucosa and muscularis mucosae in the area of involvement and permitted direct contact between the glands and the smooth muscle cells. After this contact was established the two muscular coats and the peritoneum became permeated with the infiltrating mucosal glands, resulting in a picture easily recognizable as carcinoma.

NEUROFIBROMATOUS TUMORS OF THE EARS OF RATS PRODUCED BY PROLONGED FEEDING OF CRUDE ERGOT. Arthur A. Nelson and (by invitation) O. Garth Fitzhugh and H. J. Morris, Washington, D. C.

Abstract. About 100 albino rats were fed crude ergot in dosages of 5, 2 and 1 per cent of their diet for 15 months to 2 years, with the intention of testing possible chronic toxic effects. After about 1 year, tumors, histologically neurofibromas, began appearing on the ears. Most of the animals on 5 per cent ergot, a few on 2 per cent and none on 1 per cent developed neurofibromas; they occurred only on the ears. Of several hundred similar rats of equal age treated with a variety of food and drug dyes and solvents, only 1 has developed a similar tumor, also on an ear. Tumors other than neurofibromas have occurred in both the ergot-fed and other groups with their usual spontaneous frequency. No gangrene has been caused by the ergot feeding; the alkaloid content of our dosages is probably much too small to produce gangrene. Pathological changes in the viscera have been minor. The fraction of the ergot responsible for tumor production has not yet been determined.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) I would like to ask whether any attempts have been made to transplant the tumor.

(Dr. Nelson) No.

(Dr. Arthur W. Wright, Albany, N. Y.) I would like to know whether or not Dr. Nelson has carried out these experiments in more than the one strain of rats.

(Dr. Nelson) No. Our animals were the ordinary albino Osborne-Mendel strain of rats.

(Dr. Wright) Did any tumors arise spontaneously in animals that had not received ergot?

(Dr. Nelson) Our rats have about the usual number of spontaneous tumors for their variety. Most of you will recall a paper by Curtis and

tein storage. When the epithelial lining of the benign as well as of the malignant tumors piles up and fills the lumen, the cells lose their polarity and become transformed into adrenal-cortex-like cells. The histological and cytological pictures reveal that with the loss of polarity, another latent prospective potency, the potency to differentiate into adrenal cortical elements, is awakened. These tumors consequently must be traced not to misplaced, definitely differentiated renal or adrenal cells, but to latent, prospective, neoplastic renal, or adrenal potentialities hidden in tubular cells which cannot be distinguished from normal renal cells by routine microscopy. Under the stimulus of chronic inflammation or sclerosis, the latent potentialities are aroused; the renal character is manifest first and gives rise to papillomatous formations, but later, after proliferation and solidification have eliminated cellular polarity, the adrenal cortex potency becomes manifest.

HISTOGENESIS OF EXPERIMENTAL ADENOCARCINOMA OF THE SMALL INTESTINE IN MICE. Harold L. Stewart and (by invitation) Egon Lorenz, Bethesda, Md.

Abstract. Adenocarcinoma of the small intestine was induced in mice by oral administration of aqueous olive oil emulsions of either 1, 2, 5, 6-dibenzanthracene or 20-methylcholanthrene. The growths occurred at all levels in the small intestine, the majority lying from 15 to 20 cm. from the pylorus. The tumors were composed of atypical glands derived from the intestinal mucosa. All coats of the intestine were permeated by the neoplastic tissue. Metastases occurred to the pancreas, base of the mesentery and mesenteric lymph nodes. Excised fragments from several tumors were successfully transplanted subcutaneously into mice of the same strain as the animal in which the tumors originated and have been carried for many generations without change in histologic structure. To date a large number of mice have been autopsied in which the intestinal changes occurring during the stages of development of the carcinoma could be followed in detail.

Polypoid carcinoma of the small intestine occurred rarely under these experimental conditions in contrast to the frequency with which this form of neoplasm is observed in the large intestine in man. Of intermediate frequency but still rare was the development of carcinoma at the site of a lymph follicle in the small intestine. In this location the mucosal glands grew down into the follicle and into the underlying muscle, resulting in fissures and complete destruction and disappearance of the lymphoid structure. The latter was replaced by an area of atypical glands which completely permeated the intestinal wall.

The most frequent changes observed in the intestine during the evolution of the neoplastic process were as follows: At first there was hyperplasia and downgrowth of the basal glands of the mucosa into the lamina propria. A number of glands appeared to be affected simultaneously over an area approximately 1 mm. in width. The downward growth of these glands into the submucosa did not progress by way of lymphatic vessels. Instead the lymphatic vessels in the submucosa became constricted and ultimately obliterated due to the proliferation of reticulum and collagen in the submucosa about them. The cells lining the infiltrating basal glands became more atypical and were sometimes several layers thick. Individual cells

or multiple lesions in the bones of 10 children whose progress has been followed for from 3 to 10 years since the recognition of the first lesion. These appeared to be identical with what has been described in the recent literature as "solitary granuloma" by Otani and Ehrlich, and "eosinophilic granuloma" by Lichtenstein and Jaffe, and by Hatcher. They involved mainly the flat bones, particularly the skull, ribs and pelvis, although the long bones did not escape. Roentgenological examination revealed round, oval or irregular destructive lesions often with a punched-out appearance which suggested either myeloma, tumor metastases or Schüller-Christian's disease. The presenting sign was usually either swelling or pain. Healing occurred readily under X-ray therapy or after curettage, and to some extent spontaneously. Pathologic examination revealed a granulomatous process in which eosinophilic infiltration was frequently, but not always, a prominent feature. Foci of necrosis without evidence of bacterial inflammation or suppuration were commonly present. Stimulation of the bone marrow was so marked in some instances that the diagnosis of myelocytic myeloma was excluded only with difficulty. Large mononuclear phagocytes, sometimes in giant cell formation, dominated the picture. These contained débris, remnants of destroyed bone, and often finely divided lipid which was stainable by Scharlach R, and was only rarely doubly refractile. Bacteriologic examination and a limited number of animal inoculation studies revealed no evidence of an infectious agent. No attempt has been made to isolate and identify a possible filtrable virus. Eosinophilia (6 per cent) was demonstrated only once. Blood cholesterol values were essentially normal. The total blood fat was significantly elevated in one half of the patients. Nine patients are alive and apparently in good health with no evidence of visceral disease; no details could be obtained concerning 1 patient who died outside the hospital. Comparison of the pathologic material obtained from these 10 patients with lesions in the skeleton and viscera in Schüller-Christian's disease and with several examples of what is known in the recent literature as "Letterer-Siwe's" disease, and study of recorded descriptions of the evolution of bone lesions in Schüller-Christian's disease have led us to the conclusion that all three conditions represent variations in degree, stage of involvement and localization of the same basic disease process. There is no implication in the foregoing statement that any one of these three conditions is a xanthomatous process or a manifestation of a primary alteration of lipid metabolism. These studies do not lend support to the conclusion that "eosinophilic or solitary granuloma of bone" is either a new or a separate disease entity. If this suggestion concerning the nature of these benign bone lesions is correct, caution must be exercised in prognosis because of the possibility of later visceral involvement.

Discussion

(Dr. Louis Lichtenstein, New York, N. Y.) The material which Dr. Farber has presented is very interesting indeed. However, it is difficult from his presentation to accept his interpretation of the condition in question as an expression of Schüller-Christian's disease. In fact, he did not define his criteria for the anatomic diagnosis of Schüller-Christian's disease.

Apparently, Dr. Farber holds that the finding of a few foam cells in an occasional eosinophilic granulomatous lesion establishes a link between the

Bullock reporting several hundred spontaneous rat tumors in a colony numbering thousands; our incidence is about the same as theirs, and our ergot animals have had just their share of the tumors and no more.

(Dr. Wright) Were any of these spontaneous tumors fibrous tissue tumors?

(Dr. Nelson) Of three dozen, about 6 were, and they were more of a malignant spindle cell sarcoma type, with none of the features of neurofibroma; that, of course, opens up the big question as to whether these spindle cell sarcomas originate from perineural fibrous tissue. One rat had a much more benign fibroma. Only 1 rat out of hundreds had a neurofibroma similar to those reported here and the tumor was also on an ear.

(Dr. Antonio Rottino, New York, N. Y.) What was the dosage of ergot?

(Dr. Nelson) The doses given were 1, 2, and 5 per cent of the diet; the ergot was ground in small quantities so that it would not lose its potency and mixed with the diet. It was learned that the animals would do better if the 5 per cent doses were worked up to over a period of about a month.

(Dr. James H. Peers, Washington, D. C.) I would like to ask if there was any necrosis of the ears before the tumors appeared.

(Dr. Nelson) No; in the very earliest stages the ear appeared normal, and then a very small elevation developed, but there was no necrosis or anything of that nature until after the tumors became larger.

HISTOLOGICAL CHANGES PRODUCED IN ORAL SQUAMOUS CELL EPITHELIOMAS BY FRACTIONATED EXTERNAL IRRADIATION. John W. Hall and (by invitation) Milton Friedman, New York, N. Y.

Abstract. A histological study of 28 cases of squamous cell epitheliomas treated by fractionated irradiation is presented. Each case had from 3 to 9 biopsies, including 1 before treatment. Special emphasis was placed on irradiation keratogenesis.

THE NATURE OF SO-CALLED ANGIOBLASTIC MENINGIOMAS—SCLEROSING HEMANGIOMAS OF THE MENINGES. Orville T. Bailey, Boston, Mass.

Abstract. Angioblastic meningiomas have been regarded as tumors of blood vessel origin. However, their fat and pigment content and particularly their neuroglial element have served to set them apart from other hemangiomas. In view of the consequences of sclerosis in cutaneous hemangiomas it appears that the angioblastic meningiomas are hemangiomas in which a similar process of sclerosis has occurred. The sequences of pigment and fat deposition are identical with those in the cutaneous form. The meningeal tumors extend for a distance into the underlying brain. The neuroglia in the neoplasms results from participation of the ectodermal supporting tissue in the process of sclerosis. This is confirmed by finding identical tissue sequences in hemangiomas which are located wholly within the brain substance.

THE NATURE OF "SOLITARY OR EOSINOPHILIC GRANULOMA" OF BONE. Sidney Farber, Boston, Mass.

Abstract. This report deals with results of studies, made in part with the collaboration of my clinical colleagues, Dr. William Green and Dr. Leo J. McDermott, concerning the nature of certain benign, destructive, solitary

absent in a considerable number of cases of Schüller-Christian's disease. Consequently the regrettable statement has appeared repeatedly in the literature that a biopsy in Schüller-Christian's disease is not necessary because such characteristic cells may not be found. The eosinophilia which is considered to be practically pathognomonic of eosinophilic granuloma is also frequently a very prominent feature of Schüller-Christian's disease, and the eosinophilia of the peripheral blood in cases of eosinophilic granuloma may be duplicated in many cases of Schüller-Christian's disease. The lesions in both diseases may be associated with considerable fibrosis.

I think one lesion of a xanthomatous character with which Dr. Farber is familiar, but probably did not have time to mention, is the so-called solitary xanthoma of bone, of which numerous examples exist in the literature and which can very readily be placed in the same category as so-called eosinophilic granuloma and Schüller-Christian's disease.

(Dr. Sadao Otani, New York, N. Y.) May I ask if there was any history of trauma among your cases? Most of our cases have had a definite history of trauma. One of the cases we observed was a boy who was hit on the skull by a baseball. The granulomatous lesion developed at the exact site of trauma. A biopsy of this lesion showed the typical histology. None of our cases showed lipid cells which to me are histologically characteristic of Schüller-Christian's disease.

We are fully familiar with the picture in Schüller-Christian's disease. We observed one particular case from the beginning to the end of the disease, *i.e.*, from the initial lymph node biopsy to autopsy. It was true that the biopsied lymph nodes showed practically no lipid cells; nevertheless we suggested the diagnosis of Schüller-Christian's disease because of other histologic criteria. Gradually, however, the patient began to show typical bony lesions on X-ray examination. When the patient died 9 months after the first biopsy, at autopsy we found fully characteristic lipid granulomatous lesions of Schüller-Christian's disease.

We did not overlook a similarity between the solitary granuloma and Schüller-Christian's disease. In fact, when in 1932 one of our cases of granuloma was first observed, Dr. Klemperer seriously considered that the lesion might belong to those seen in Schüller-Christian's disease. None of our cases, however, showed lipid cells and they pursued clinical courses different from the cases of Schüller-Christian's disease. Therefore, the idea that the granulomatous lesions might belong to the lipid granulomatosis group was given up by us, and we finally came to the conclusion that we were dealing with a discrete granulomatous lesion whose cause is thus far unknown. One of the cases we reported did not reveal conspicuous numbers of eosinophilic leukocytes in the lesion, although it was otherwise typical of this granuloma. Therefore, we prefer the term "solitary granuloma" to "eosinophilic granuloma."

(Dr. Farber) To answer Dr. Otani first concerning the relation of fracture to the lesion, I may say that trauma of importance was found in the history of several cases. In one instance a boy was struck on the head by a ball, and X-ray examination showed a punched-out defect in the skull which had been there obviously for some time antedating the injury.

I am very much interested in the observations of Dr. Gross. I certainly add solitary xanthoma to this group of lesions, although I did not mention it. We have had no evidence of visceral lesions in the group of 10 patients I

latter condition and Schüller-Christian's disease. We have had the opportunity of examining biopsy material from 12 cases of eosinophilic granuloma. (Incidentally, all but 1 of these showed only a single bone lesion.) None of the cases which we have seen showed collections of foam cells as a prominent feature of the lesion, and altogether the cytology of the lesion is not the cytology regarded as characteristic for Schüller-Christian's disease. One observes areas of focal necrosis in the lesions of eosinophilic granuloma. Furthermore, in most of the lesions, there is very appreciable and often heavy infiltration of eosinophilic leukocytes, which may be so concentrated as to simulate micro-abscess formation. Moreover, the macrophages which constitute another conspicuous feature of the lesion, as Dr. Farber has emphasized, contain phagocytosed red blood cells and eosinophiles, iron pigment and eosinophilic granules. That is, they are not essentially lipophilic macrophages, and while some may take up a very slight amount of lipoid granules, many contain none.

Clinically, too, eosinophilic granuloma is different from Schüller-Christian's disease. The former sets in abruptly, sometimes with a mild febrile reaction, and a good percentage of the patients also present an eosinophilia ranging between 4 and 10 per cent. The bone lesions develop rapidly and large destructive lesions may already be present within a few weeks. In cases with multiple lesions, most of these lesions seem to appear all at once, like a shower, which they seem not usually to do in Schüller-Christian's disease.

(Dr. Henry L. Jaffe, New York, N. Y.) I want to ask Dr. Farber whether any of the cases he described showed involvement of the dura, pleura or periosteum — features which are supposed to be prominent in the pathologic anatomy of Schüller-Christian's disease. It seems worth while to cite here, as a piece of evidence against Dr. Farber's conception of eosinophilic granuloma of bone as a form of Schüller-Christian's disease, the case of a girl 8 years of age now under observation at our hospital. Six weeks prior to admission she complained of moderate generalized abdominal pain and when she was permitted to be out of bed (a week later) it was noted that she had a mild limp on the right side. X-ray examination of the right hip region 1 week later showed an area of rarefaction in the neck of the right femur about $\frac{3}{4}$ of an inch in diameter. This lesion increased rapidly in size, and additional lesions have been found in the following sites: shaft of left femur, ninth right rib, and second and ninth left ribs. While in the hospital she has shown a leukocytosis and a mild febrile reaction. Two lesions have been explored, and the curetted tissue revealed a picture identical with that which Dr. Lichtenstein and I had previously described under the heading of "eosinophilic granuloma of bone." The lesions did not show collections of foam cells, fibrous tissue reaction to the lesion, walling-off reaction of the bone around the lesions, or any other feature which pathologists in the past have come to recognize as belonging to the pathologic anatomy of Schüller-Christian's disease. I cannot understand how a condition can be held to represent Schüller-Christian's disease (even as a variant) when it has a totally different pathologic anatomy and clinical course.

(Dr. Paul Gross, Pittsburgh, Pa.) I should like to say that from a study of this condition, of which we have one example, and from a study of the literature, Dr. Harold Jacox and I have come to a conclusion identical with that given by Dr. Farber. The foam cells which in many cases of Schüller-Christian's disease have been stressed as important diagnostically, have been

than might be suspected from the apparent rarity of the *in situ* lobular form of the disease.

EARLY CANCER OF THE GASTRO-INTESTINAL TRACT. William Carpenter MacCarty, Sr., Rochester, Minn.

Abstract. From 1917 to 1940 inclusive, I have routinely measured all resected malignant and benign tumors. I believe it is fair to associate earliness and lateness with size and the presence or absence of involvement of lymph nodes. This review deals only with cancers of the stomach and large intestine because they are among the most frequent sites of cancer. I have arbitrarily taken 2.5 cm. in diameter as the criterion of smallness or earliness. Table I is a summary of data originally determined for each of the years, but condensed here so as to include only the totals for the 24 years.

TABLE I
Small Cancers (2.5 cm. in Diameter or Under)

With lymphnodal involvement			Without lymphnodal involvement		
Stomach	Rectum, sigmoid, rectosigmoid	Rest of colon	Stomach	Rectum, sigmoid, rectosigmoid	Rest of colon
52 of 1162	27 of 1140	6 of 322	141 of 834	106 of 1683	11 of 433

TABLE II
Summary of Measurements of 6474 Resected Cancers

	Stomach	Rectosigmoid, rectum, sigmoid	Rest of colon
Number	2408	3102	964
Average size	6 cm.	5.7 cm.	7 cm.
Percentage with lymphnodal involvement	62	37	38
Largest	19 cm.	20 cm.	15 cm.
Smallest	0.5 cm.	0.9 cm.	1 cm.
Percentage of all 2.5 cm. in diameter or under	9.7	4.7	2.2

Summary. The statistics suggest that there has been some improvement in recognition of early cancerous lesions by the general medical profession. The greatest improvement is seen in the stomach, the rectum and rectosigmoid, all of which regions are now becoming generally accessible to more frequent direct vision and X-ray study. The large intestine from the ileum to the rectosigmoid is still a region for roentgenoscopic improvement, although the figures show that there has been a probable improvement.

READ BY TITLE

UNILATERAL RENAL ATROPHY AND HYPERTENSION. A. B. Baggenstoss and (by invitation) Nelson W. Barker, Rochester, Minn.

Abstract. This study includes all cases (84) of unilateral renal atrophy which have come to necropsy at the Mayo Clinic. There were 48 cases of pyelonephritic atrophy, 28 cases of hydronephrotic atrophy and 8 cases of pyone-

described today. In 1 patient who has had twenty-five bone lesions in the last 10 years, a yellowish nodule appeared on the leg several months ago. This, on histologic examination, showed much the same picture that was described today.

For many years we experienced difficulty in interpreting the lesion. It was not until transitional stages were encountered that the nature of the process became clear. I believe one difficulty is that we have too narrow a conception of Schüller-Christian's disease. This might well be expected since the disease picture was described originally on roentgenological and medical grounds and not on the basis of pathological examination. I might add in passing that I am not willing to accept the classification of Schüller-Christian's disease in the group of lipid metabolic diseases. In regard to the problem at hand we have been forced to the conclusion, on the basis of pathologic studies and information gained from the clinical and roentgenological characteristics of the disease process, that eosinophilic granuloma and lipo-granuloma are but variations of the same process, and that eosinophilic or solitary granuloma of bone, Schüller-Christian's disease and Letterer-Siwe's disease differ from one another only in the degree and site of involvement and the duration of the process. Further studies will be necessary before the etiologic factor can be defined.

LOBULAR CARCINOMA IN SITU—ONE OF THE RARE FORMS OF MAMMARY CANCER.* Frank W. Foote (by invitation), New York, N. Y.

Abstract. Mammary carcinoma *in situ* has been recognized for many years, but the term, *in situ*, has not been used. This long-recognized form has been designated "noninfiltrating comedo-carcinoma." The type of mammary carcinoma to be presented here differs from this type in that it takes origin not from the larger duct system but from the component parts of the mammary lobule.

The lesion in the pure, noninfiltrating form is quite infrequent, only 2 such examples being encountered in a consecutive series of 300 primary operable mammary cancers. There is no way in which a clinical diagnosis of lobular carcinoma *in situ* can be made. Even in excised breast tissue, gross features of mammary cancer are missing and one must depend upon microscopic examination for diagnosis. The microscopic changes include an abrupt alteration in lobular cytology featured by the appearance of "pagetoid" cells. The lesion occurs in multiple lobules that may be several centimeters apart, and hence when this pattern is found it is hazardous to stop treatment short of simple mastectomy. One case is cited in which local excision was done. The significance of the histology was not appreciated and within a few months this patient had infiltrating mammary cancer with metastases to axillary nodes and skeleton. How long lobular mammary carcinoma may remain *in situ* cannot be definitely stated, but its duration for 1 year without infiltration has been seen in 1 case. As soon as infiltration occurs, the gross appearance of lobular carcinoma becomes similar to that of many infiltrating mammary carcinomas. Microscopically, however, the pattern of infiltration is characteristic and easily recognized after some practice. The discovery of this type of growth pattern in a good many mammary cancers promotes the belief that infiltrating lobular mammary carcinoma is more frequent

* This article appears in full in this issue. See p. 491.

is reported. The occlusion of the portal system was by calcific thrombi and probably resulted from acute appendicitis. The aneurysm of the splenic artery was of the cirroid type, the vessel measuring 1.5 to 2 cm. in diameter and 55.5 cm. in length when it was straightened. The hepatoma was associated with portal cirrhosis.

THE PRESENT INCIDENCE OF TUBERCULOUS INFECTION. William H. Carnes (by invitation), New York, N. Y.

Abstract. The prevailing concepts of the pathogenesis of adult tuberculosis are based on the fact, established in the early part of this century, that virtually every city-dwelling adult has at some time acquired a tuberculous infection. That this is still a fact in the United States today has been questioned on the strength of the results of tuberculin surveys on large bodies of students in various communities in the past 10 years. These suggest that perhaps as much as half the population now reaches adult life without having acquired an infection. However, with the recently accumulated evidence that the tuberculin test may fall far short of establishing the true incidence of infection, it becomes desirable to gain more certain information from a postmortem anatomical search. Such a survey has been made on cases autopsied at the Baltimore City Hospitals and the Johns Hopkins Hospital in the years 1938 to 1940. The series includes only patients dying of diseases other than tuberculosis in which the entire lungs, together with all the bronchial and tracheal lymph nodes, were available for detailed search with the aid of the X-ray. All lesions were identified grossly and all were examined microscopically unless perfectly typical. The criteria for the diagnosis of their tuberculous origin were the same as those used in similar reported investigations in the past. The results of the routine autopsy examination, including that of the mesentery, were also utilized. A total of 536 cases was examined. Of these 307 were white and 229 negro. There was a fairly equitable distribution of the cases over the various age groups from 2 months to 89 years.

The results show that, as in the past, there is a rapid rise in the proportion of infected individuals from infancy to adult life. The greatest increment in the percentage of infected individuals occurs in the period from 5 to 15 years of age. During this period approximately 40 per cent of the population sampled acquired a tuberculous infection. The results also indicate that probably less than 30 per cent of Baltimore's population reaches the age of 20 years without having had an infection. All of 114 individuals over 60 years of age showed evidence of infection. No significant differences were revealed between the white and negro groups in any of these respects.

A comparison of these data with the results of a similar investigation by Opie in St. Louis in 1916 has been attempted. Serious limitations are imposed by the paucity of cases in several age groups of that study. However, when those results are examined by the same standards as the contemporary data, it appears probable that in the population examined by Opie not more than 15 per cent reached the age of 20 years without having acquired an infection. The approximate nature of all these estimates must be emphasized. Convincing proof that the incidence of tuberculous infection in St. Louis in 1916 was different from that in Baltimore in 1940 is lacking. No support is offered for the thesis that the incidence of tuberculous infection in city-dwelling adults has changed drastically in the past 20 years.

phrotic atrophy. Fourteen cases of unilateral hypoplasia also were studied. Death in most of these cases was due to neoplastic disease or infections of various types. Hypertension was a cause of death in only 5 cases. The incidence of hypertension was determined for the different types of renal atrophy and also for a control group of 100 consecutive cases of similar age distribution in which necropsy was performed. The incidence of hypertension in this latter group was 29 per cent. Only in the cases of pyelonephritic atrophy (39.6 per cent) and pyonephrotic atrophy (37.5 per cent) was the incidence of hypertension greater than in the control group. The incidence of hypertension was 41.9 per cent for the cases of pyelonephritic atrophy in which the atrophied kidney weighed 75 gm. or less and 35.4 per cent for the cases in which the atrophied kidney weighed between 75 and 110 gm.

In 26 of the 56 cases of pyelonephritic or pyonephrotic atrophy there was some degree of active inflammation in the atrophic kidney. In the scars of the kidneys in which there was pyelonephritic or pyonephrotic atrophy, there was generally arteriosclerosis grade 2 or 3 (on the basis of 1 to 4, in which 1 designates the mildest and 4 the most severe condition) while in the non-scarred portions, when these were present, the arteries were as a rule normal or revealed less sclerosis than those in the scars. This was true of the cases in which there was hypertension as well as those in which there was not hypertension.

In 14 of the 56 cases in which there was pyelonephritic or pyonephrotic atrophy, there was some degree of active inflammation in the opposite kidney. Inflammation in the opposite kidney was more often present in the cases in which there was hypertension than in those in which there was not hypertension. The degree of arteriosclerosis in the opposite kidney was generally less severe than in the atrophied kidney and in 10 of the 22 cases in which pyelonephritic or pyonephrotic atrophy was associated with hypertension the arteries and arterioles in the opposite kidney were considered normal for the age of the patient.

Although the results are of questionable statistical significance because of the small number of cases, they suggest that unilateral pyelonephritic or pyonephrotic atrophy is associated with hypertension more often than one would expect on the basis of chance. They also suggest that hypertension is more likely to be present if the degree of atrophy is severe.

BLASTOMYCOSIS: SPREAD AND TISSUE REACTION IN THE HUMAN. Roger D. Baker, Durham, N. C.

Abstract. Twenty-two cases of blastomycosis from which histopathological material was available, including 4 complete autopsies, were analyzed with regard to the primary infection, spread of the infection in the body, and tissue reaction. The tissue reaction is contrasted with that of tuberculosis and analyzed in relation to iodide therapy, reactions to skin and complement fixation tests, and extent and duration of the disease.

CHRONIC PORTAL OCCLUSION WITH ANEURYSM OF SPLENIC ARTERY AND CARCINOMA OF LIVER (HEPATOMA). Milton D. Bosse and James M. Strang (by invitation), Pittsburgh, Pa.

Abstract. Chronic portal occlusion associated with aneurysm of the splenic artery and carcinoma of the liver (hepatoma) in a white male, 58 years old,

hemolysin and complement-fixation titers were determined in lymph of regional and opposite lymph nodes and blood serum. These titers were compared with the number of lymphocytes in the lymph and with the weight and histologic appearance of the popliteal lymph nodes. The results of these experiments support the concept that antibodies are formed in lymph nodes and that lymphocytes play a considerable rôle in antibody formation.

CYSTIC FIBROSIS OF PANCREAS. I. H. Erb, Toronto, Canada.

Abstract. This communication is concerned with cystic and fibrous changes in the pancreas, usually of young infants, associated with some type of pulmonary infection, in many cases of the nature of bronchiectasis. In some instances there is also metaplasia of bronchial epithelium.

EXPERIMENTAL STAPHYLOCOCCIC PNEUMONIA IN RABBITS. Istvan A. Gaspar, Rochester, N. Y.

Abstract. In order to study experimental lung lesions produced by staphylococci, a series of rabbits was injected intratracheally with virulent *Staphylococcus aureus*. A 5 cc. saline suspension of a 24-hour growth of hemolytic *Staph. aureus* (2500 million organisms per cc.) was injected into each rabbit. One rabbit was killed every day up to the 12th day, then 1 on the 16th day and on the 20th day. The rabbits developed tracheitis, bronchitis and bronchogenic lung infection very similar to that observed in human lungs. The infection did not kill the rabbits. The initial hemorrhagic consolidations were followed by small and large gray consolidations and by small abscesses seen microscopically. The consolidations involved one third to one half of each lung. Staphylococci were recovered from the lungs in pure culture up to 8 days after injection. After that day the lung cultures became completely negative. Masses of staphylococci were seen in the lungs microscopically during the first days after the injection. They disappeared gradually. The small abscesses became absorbed later and by the 20th day only congestion, some fibrosis and the final stages of repair were present. These experiments showed that although the rabbit lung appears to possess better resistance to *Staph. aureus* than the human lung, nevertheless they demonstrate that *Staph. aureus* can cause bronchogenic pneumonia and lung abscesses without the association of any other organisms or virus. It is hoped that the action of some of the new drugs on *Staph. aureus* pneumonia can be tried with similar experiments.

Sterile saline and *Staph. aureus* vaccine were also injected intratracheally in other rabbits to determine the lung pathology produced by these agents.

GLOMERULONEPHRITIS OF RATS FOLLOWING THE ADMINISTRATION OF SULFAPYRIDINE. Paul Gross and (by invitation) Frank B. Cooper and William A. Morningstar, Pittsburgh, Pa.

Abstract. Relatively large doses of sulfapyridine were administered by stomach tube daily for 17 to 67 days to white rats from which one and one-half kidneys had been removed. Similar medication was also given to a group of unilaterally nephrectomized rats and to a group of normal, unoperated rats. The gross and microscopic pictures of urolithiasis medicamentosa with hydronephrosis or hydro-ureters were encountered in only 20 per cent of all animals in the three series.

EFFECTS OF THE CONTINUED ADMINISTRATION OF SULFATHIAZOLE AND SULFAPYRIDINE TO MONKEYS. David R. Climenko (by invitation) and Arthur W. Wright, Albany, N. Y.

Abstract. Sulfathiazole and sulfapyridine, in doses from 0.5 gm. per Kg. per day up to 10 gm. per Kg. per day, were administered to monkeys for a maximal period of 28 days in order to obtain some evidence of the comparative toxicity of these two compounds. Drugs were administered by stomach tube as milk suspensions at 8-hour intervals throughout the entire period of medication. Daily observations of blood concentrations were made.

At a dose level of 0.5 gm. per Kg. per day, animals receiving sulfapyridine died on the 13th, 14th and 24th days of medication, respectively. Hematuria was present in all. At autopsy, urolithiasis, degenerative changes of the tubular epithelium, particularly of the collecting tubules, pyelitis and cystitis were observed. Monkeys receiving the same dose of sulfathiazole showed no ill effects during the 28 days of medication. One animal of this latter series, sacrificed on the 29th day for necropsy, showed no significant pathological changes other than slight edema of the kidney and a chronic inflammatory process of the renal pelvis.

The difference between sulfathiazole and sulfapyridine disappeared when the dose level was increased; at and above 1.0 gm. per Kg. per day fatalities occurred and severe renal lesions were observed in both series. These manifested themselves as parenchymatous and fatty degenerative changes of the epithelium of the convoluted and collecting tubules associated with the presence of crystalline material in the latter; focal necrosis and focal inflammation, and ulceration, necrosis, and desquamation of the epithelial elements of the larger collecting tubules. The renal pelvis showed acute inflammatory reactions associated with submucosal hemorrhages. The severity of the lesions varied directly with the height and duration of the concentration of the drug in the blood. It should be pointed out that the dose range employed in this series approximates 10 to 200 times the usual therapeutic range.

BRAIN CHANGES IN PERTUSSIS. Vera B. Dolgopol, New York, N. Y.

Abstract. Neurological complications occur in pertussis in small children and are fatal in a large proportion of cases. The most frequent histologic finding is the "eosinophilic" (ischemic) degeneration of hippocampal pyramids and of Purkinje cells. Multiple scattered hemorrhages and lymphocytic plugs in capillaries and veins are next in frequency. No perivascular cuffing is observed. Loss of myelin is seen very rarely, apparently as a result of compression and secondary degeneration of myelin within areas of perivascular hemorrhages. The process is not an encephalitis, but rather an encephalopathy. No *Haemophilus pertussis* or virus was found in several brains examined. The pathogenetic basis for the brain lesions in pertussis, according to several authors, is stasis in the cerebral circulation.

THE PRODUCTION OF ANTIBODIES IN THE POPLITEAL LYMPH NODE OF THE RABBIT. W. E. Ehrich and (by invitation) T. N. Harris, Philadelphia, Pa.

Abstract. Various antigens were injected subcutaneously beneath the plantar surface of the foot, and after various intervals lymph was collected from the single efferent lymph vessel of the popliteal lymph node. Agglutinin,

THE TITRATION OF TRACES OF ANTIBODY: A TECHNIC USING MAXIMAL SERUM PROPORTIONS WITH SECONDARY INDUCTION OF AGGREGATION.
Herbert Lund (by invitation), Boston, Mass.

Abstract. A marked increase of sensitivity can be obtained by treating each of the two stages of serological aggregation as individual reactions. This is done by adjusting the proportions of serum and antigen to favor maximal sensitization (a maximum of serum and an arbitrary minimum of antigen) and secondarily inducing aggregation of the diluted antigen (by centrifugation and reconcentration). This differs from the usual methods which strive to accommodate the conditions of both stages simultaneously by using a single ("optimal") proportion of serum and antigen. The method was applied to the iso-agglutination reaction and (with the modification of lowering specific gravity by the addition of saline solution prior to centrifugation) to the flocculation test for syphilis. The sensitivity of these reactions was increased 32 to 64 times that of standard technics. The main quantitative relationships found were as follows:

1. Within a wide range, sensitivity is directly proportional to the amount of serum and inversely proportional to the amount of antigen used in the reacting system. There is no optimal zone.

2. Volume increase by the addition of saline diluent does not appreciably affect the combination of antigen and antibody. Only in the extremely large volumes is the efficiency of the reaction appreciably affected.

3. From the above direct proportion, a convenient method of expressing titrations is evolved. This is to state the calculated minimal volume of undiluted serum required to aggregate an arbitrary unit of antigen. This can be determined by using the above technic of maximal serum proportions and quantitatively titrating by progressive serum dilution, and can be calculated by the formula: volume of serum used times the dilution at the end-point divided by the arbitrary units of antigen used in the reaction.

By a preliminary clinical trial of the above method it was found that occult reagin could be detected and titrated in sera of latent and treated syphilitic patients and in the sera of many normal individuals.

THE FATE OF TUBERCLE BACILLI PHAGOCYTED IN VIVO AND IN VITRO BY MONONUCLEARS DERIVED FROM NORMAL AND IMMUNIZED RABBITS.
Max B. Lurie, Philadelphia, Pa.

Abstract. A mixture of tubercle bacilli and India ink was injected intravenously into normal and tuberculous rabbits. Two days later, bone marrow containing both phagocytized bacilli and carbon particles was removed from each rabbit. Each specimen of bone marrow was divided into two portions one was cultured to determine the number of living bacilli present, the other portion was placed in the anterior chamber of the eye of a normal albino rabbit. The marrow derived from the normal rabbit was placed in one chamber; the cells from the immunized animal were placed in the other chamber of the same rabbit. Two weeks later the irides of both eyes with their growth of carbon-bearing mononuclears were removed and cultured. It was found that within the mononuclears derived from the normal animal the bacilli grew abundantly, while within those derived from the tuberculous animal the growth of bacilli was definitely inhibited, in spite of the fact that these cells were growing in a nonimmunized environment.

A nonexudative glomerulonephritis was found in some rats. This was most frequent and severe in the animals from which one and one-half kidneys had been removed, while the unoperated animals exhibited the least involvement and relatively slight or early lesions. The unilaterally nephrectomized animals occupied a position intermediate in amount and severity of renal involvement. The glomerular lesions consisted of thickening of basement membranes of glomerular capillaries, hyaline degeneration, focal necrosis, endothelial proliferation of glomerular tufts and focal adhesions of tufts to Bowman's capsule in foci of capsular epithelial proliferation.

Sulfapyridine did not seem to be the direct cause of these lesions. It appears more likely that the production of the lesions was related to the excessive work required of the glomeruli which remained after surgical resection of renal tissue and the consequent obstruction by sulfapyridine uroliths. The glomerular lesions observed resemble those caused by nephrotoxic sera or by high protein diet.

ENVIRONMENTAL FACTORS INFLUENCING FEVER IN PULMONARY TUBERCULOSIS: A STATISTICAL STUDY. John S. Howe and (by invitation) Alvin Mayne, Richmond, Va., and Chicago, Ill.

Abstract. The percentile occurrence of fever in the population of a tuberculosis sanitarium was determined daily for a period of 14 months. Seasonal, weekly, and daily trends were identified and correlated statistically with various environmental factors, such as visiting days, the environmental temperature, the barometric pressure, etc. The results and their significance are discussed.

EXPERIMENTAL COLLOID DROPLETS IN RENAL EPITHELIUM. Frederick Johnson (by invitation) and Hans Smetana, New York, N. Y.

Abstract. The kidneys of urodeles have two types of nephrons: (1) "closed" nephrons which are similar to those in mammals; (2) "open" nephrons which communicate with the peritoneal cavity by means of the "nephrostomial canal" which is lined by ciliated epithelium and opens into the proximal portion of the convoluted tubules. Materials injected into the peritoneal cavity reach the kidney tubules of the "open" nephrons without passing through the glomerular filter; the "closed" nephrons can be used as controls.

Various proteins—casein, serum albumin and globulin, egg albumin—were injected into the peritoneal cavity of *Salamandra punctata* and *Necturus*, and were found to be taken up by the tubular epithelial cells of the "open" nephrons in the form of colloid droplets. None was present in the "closed" nephrons. The identity of the colloid droplets with the injected material was insured by coupling the various proteins with the disodium salt of 2 naphthol-3:6 disulfonic acid which has an intense red color.

Likewise the injection of fats and lipids was followed by fatty changes and the presence of cholesterol crystals in the renal epithelium of the "open" nephrons. The colloid droplets and fatty changes thus produced in renal epithelial cells were in no way different from those seen in nephrosis.

It is concluded that the colloid droplets and fatty changes seen in human nephrosis are due to reabsorption and storage of proteins and lipids passed through the glomerular filter into the tubules.

cases by one of us (B. P.), these are the only cases recorded in the English literature which were proven by the demonstration of the specific micro-organism.

THE METEOROGENESIS OF CEPHALIC MALFORMATIONS. William F. Petersen and (by invitation) A. Mayne, Chicago, Ill.

Abstract. Cephalic malformations may be caused either by recessive genetic or environmental factors effective during the earliest stages of differentiation. Presumably most such malformations are associated with a delay in the separation of the medullary plate and the notochord (Bonnievie). This may in turn be associated with the trend toward femaleness revealed by such malformation. In America malformations are more frequent in the northern tier of states and in adjacent states the annual trend-of-production curves reveals a high correlation coefficient.

An examination was made of the environmental conditions (temperature, barometric pressure, etc.) existing at the presumptive conception period of more than 1,000 cephalic malformations studied in the Chicago region. In general there appeared sufficient differences at various levels of temperature and barometric pressure in the frequency of malformation and of the total birth population at the same level or at least 1 day during the interval when conception presumably occurred to conclude that real differences did occur. Increases in malformations over the expected number occurred at just below normal temperatures in winter, spring and summer periods. In the autumn an excess of malformations occurred with great deviations from normal temperatures, whether positive or negative.

A RARE MALIGNANT TUMOR OF THE THYROID WITH POSTMORTEM FINDINGS. S. H. Polayes, Brooklyn, N. Y.

Abstract. G. C., a white male of about 80 years of age, had a thyroidectomy performed at the Cumberland Hospital of Brooklyn for the removal of a thyroid mass which had existed for a period of about 35 years and which, in the last 5 years, had enlarged rapidly. The mass was situated in the anterior portion of the neck and had caused difficulty in breathing, hoarseness and pronounced loss of weight, despite increased appetite. The heart was enlarged and fibrillated. The basal metabolic rate was -22 . The blood examinations, including count, chemistry and serology, were all normal.

The resected tumor measured 23 by 10 by 7 cm. and was lobulated and partially encapsulated. It was firm and its tissue was yellow-gray and dense, with a tendency to the formation of small cystlike spaces and to calcium deposition. Pathologically the tumor was considered a spindle cell sarcoma originating in a fibro-adenoma of the thyroid. The patient made an uneventful recovery and was discharged from the hospital on the 18th postoperative day. The clinical diagnoses were as follows: tumor of thyroid (sarcoma or adenocarcinoma), arteriosclerotic heart disease, left indirect inguinal hernia, hypertrophy of the prostate with diverticulum of the bladder.

Five months later the tumor recurred to its original size and this mass had to be resected again. It measured 20 by 15 by 8 cm. and presented gross and microscopic features similar to those described in the previous mass. The patient died several hours after operation. The postmortem examination revealed, in addition to the sarcoma described above, acinar metas-

Mononuclears derived from sterile pleural exudates of normal and tuberculous animals were permitted to phagocytize tubercle bacilli and carbon particles *in vitro* in the presence of normal or immune serum. The fate of these bacilli was determined by again using the anterior chamber as an incubator for the cells that had ingested the bacilli. It was found that under these clearly defined conditions the mononuclears originating in an immune animal possess in themselves greater inhibitory properties on the growth of tubercle bacilli than cells derived from a normal animal. The addition of immune serum to normal cells or of normal serum to immune cells, under the conditions of this experiment, did not significantly change their inherent properties to influence the growth of bacilli within them.

CARCINOMA OF THE PARATHYROID GLAND. Karl A. Meyer (by invitation) and Alex B. Ragins, Chicago, Ill.

Abstract. A rare case of primary carcinoma of the parathyroid gland with disseminated fibrocystic disease of the bones is described. The extensive roentgenological and biochemical study is confirmed by autopsy. The influence of the carcinoma of the parathyroid on calcium and phosphorus metabolism is discussed.

MEAT EXTRACTIVES AND THE NONPROTEIN NITROGEN OF THE BLOOD. E. Mylon (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. The ingestion of boiled ground beef, from which the fluid has been drained, causes a sharp elevation of the blood nonprotein nitrogen. This reaches the normal again only after 48 hours. When the partially evaporated fluid as well as the boiled beef are fed, the rise in blood nonprotein nitrogen is even less than after the same nitrogen content is fed in the form of raw ground meat. The meat extractive seems to contain essentials for the synthesis of the blood nitrogen.

SPECIFIC LESIONS OF THE SMALL INTESTINES IN CONGENITAL SYPHILIS. TWO ADDITIONAL CASES. Bjarne Pearson and Emil Palik (by invitation), New Orleans, La.

Abstract. Two cases of congenital syphilis involving the small intestine were observed among 1855 individuals of 1 year of age or less on whom necropsies were performed between January 1, 1937 and December 31, 1940. The lesions are bright yellow, annular plaques with occasional, superficial, central ulceration, and they are separated by varying intervals of normal intestine. Their average width is from 1 to 1.5 cm. Coalescence of the plaques to involve wider segments of the bowel can be seen. The intestinal wall beneath the plaque is thick and the serosa is covered with fibrin, causing loops of bowel to adhere to one another. A peritonitis may thus be present without perforation.

In the earlier lesions microscopic sections show mainly fibroblastic proliferation with foci of polymorphonuclear cells, lymphocytes and plasma cells in varying degrees. These "abscess-like, miliary" foci are constantly present in the intestinal lesions. In the more advanced lesions, section shows necrosis of the mucosa and predominant fibroblastic proliferation. Many *Treponema pallidum* were demonstrated in sections stained by the Levaditi and Steiner methods. As far as we know, with the three previously reported

bolisms have to be considered a prerequisite in the pathogenesis of miliary abscesses. In 1 case (Banti's syndrome) a very late stage of anoxic spots could be seen in the nucleus caudatus. This late stage was represented by a perivascular glial scar with numerous fibril-producing astrocytes and by a complete loss of nerve cells. Occasionally anoxic spots may develop secondarily into definite softenings with mobile, compound granular fat cells. However, this late stage is seen only when the anoxic area surpasses its limited perivascular extension. Smaller anoxic spots are often seen with only the four characteristics mentioned and without any tendency for the mobile type of degeneration. Early changes may be considered reversible.

The problem of whether the morphological pictures described herein are due to a lack or deficiency of oxygen (so-called anoxia) or to a deficiency of other nutritional blood elements (dextrose, minerals, etc.) cannot be solved by morphological investigation alone. However, animal experiments and physiological data conform best with the conclusion that anoxemia with consequent tissue anoxia is the main factor in causing the characteristic morphological appearance in the central nervous system.

OBSERVATIONS ON THE DISTRIBUTION OF EXPERIMENTAL ATHEROMAS IN THE ARTERIES OF RABBITS. Sigmund L. Wilens, New York, N. Y.

Abstract. This communication describes an attempt to show that intimal lipid deposits found in areas of arteries, immobilized by being enclosed in silver cuffs, after chlorestero feeding has been instituted, are not due to the injury provoked by the cuff, but rather to the aggregation (or migration) of lipid from surrounding points in the intima to the immobilized zone.

METASTASES OF PRIMARY CARCINOMA OF THE BREAST. Otto Saphir, Chicago, Ill.

Abstract. The sites of metastases of 43 breast carcinomas are given. Special emphasis is placed on the occurrence of metastasis to the spleen, suprarenal glands and ovaries, which organs were involved 10, 19 and 7 times respectively. From the microscopic appearance of the primary tumor, no conclusion could be drawn as to the length of survival of the patients after radical mastectomy or the extent of metastases at the autopsy. However, the presence of isolated tumor cells regardless of the type of carcinoma, separated from basic structures of the carcinoma, indicates a high degree of malignancy. Emphasis is placed upon small and clinically unnoticed carcinomas of the breast which may give rise to widespread metastases. In this series there was no apparent difference in the survival period of patients with and without postoperative radiation treatment.

CARCINOMA OF CERUMINOUS GLAND. Shields Warren and Olive Gates, Boston, Mass.

Abstract. One case of adenocarcinoma of ceruminous glands is reported. It developed behind the right ear of a man, 78 years old. There was an ulcer 1.2 cm. in diameter. The tumor was 2 cm. in diameter and one part was cystic. On microscopic examination the cells showed a reticulated, foamy and finely granular cytoplasm. Fine droplets of fat were present. It somewhat resembled a carcinoma of the apocrine glands. One sure and 2 probable cases of adenocarcinoma and 2 adenomas of ceruminous glands have been reported previously.

tases to the mediastinal and subpleural lymphatics as well as several interesting subsidiary findings. In addition the patient had hypertrophy of the prostate (adenomatous), dilatation of the urinary bladder (obstructive), multiple diverticula, adenomatous polyps of the gallbladder, cholelithiasis, adenomatous polyp of the rectum and fibroma (fibrosarcoma?) of the duodenum.

Representative sections of both thyroid masses were studied by the following pathologists: Drs. Douglas Symmers, Arthur Purdy Stout, Paul Klemperer, Allen Graham, Shields Warren, N. Chandler Foot, James Ewing, W. G. MacCallum and A. E. Hertzler. Almost all of the above pathologists concurred in the diagnosis of sarcoma. One called it a carcinoma; another called it a carcinosarcoma, and another did not commit himself.

MORPHOLOGIC APPEARANCE OF CEREBRAL ANOXIA. Gabriel Steiner, Detroit, Mich.

Abstract. Cerebral anoxia has four morphological characteristics: (1) pallor of the involved tissues, particularly of the cerebral intercellular substance, seen in hematoxylin and eosin and other stainings; (2) sharp demarcation of these anoxic areas against the adjacent tissues; (3) well-defined perivascular arrangement of anoxic areas or at least definite regional relationship to the vascular bed; and, negatively, (4) the absence of visible change of the vascular walls and the absence of inflammatory or glial reactions. The nerve cells may show an ischemic lesion, or they may show no detectable lesions. These findings suggest beginning necrobioses.

In the routine examination of 2,000 brains, I found 12 cases having anoxic spots as incidental findings. The 12 cases can be divided into three groups. The first group of 3 cases represents mechanical interruption of the blood supply either by ligation or by plugging of vascular lumina (1 case of ligation of the internal carotid after fracture of the mandible, 2 with capillary bacterial embolisms). The second group consists of 5 cases with marked generalized anemia (2 cases with lymphatic anemia, 1 with thrombocytopenic purpura, 1 with Banti's syndrome, 1 with carcinoma of the cervix with severe anemia). The third group consists of 4 cases of endogenous or exogenous toxic conditions (2 cases with uremia, 1 with eclampsia, 1 case in which death was due to general anesthesia). Not included in these groups are a number of cases of asphyxia found in a special investigation of the newborn, cases in which identical pictures of cerebral anoxia were seen.

The morphological criteria are well defined and specific; there are no lesions which are confusing. In the so-called spongy state of the cortex, which could be mistaken for an anoxic spot, the lesion is more diffuse; there is a coarse meshwork consisting of a dense network of glial fibers and large round or oval holes. The arrangement in the spongy state is laminar or pseudolaminar whereas the anoxic spots show a perivascular arrangement. One cannot expect to see anoxic spots until a certain length of time has elapsed. Twelve hours to several days after the causative damage are necessary. Particularly interesting are the pictures of bacterial embolisms. The first phase is indicated by bacterial masses plugging the capillary lumina and by strictly perivascular anoxic spots. The second phase is represented by leukocytic and bacterial diapedesis into these spots and the third phase by miliary or submiliary abscesses. Anoxic spots in cerebral bacterial em

these same rabbits during the same daily period, following single or repeated control injections of 5 cc. of sterile sodium chloride solutions of equivalent concentrations showed no neutrophilic "left shifts." These "left shifts" were due to increases in the numbers of more immature neutrophils in the circulating blood, probably through release of these cells from the bone marrow. Although the nonfilamented neutrophils ranged from 70 to 90 per cent with maximum "left shifts," metamyelocytes very rarely were observed, and cells more immature than metamyelocytes never were found in the circulating blood.

These results seem to indicate that some substance or substances, not sodium chloride, present in supernatant fluid fractions of peritoneal exudates of sterile rabbits produced neutrophilic "left shifts" when injected intravenously into other rabbits. Following repeated injections of supernatant fluid there was an apparent "summation" of these "left shifts."

CENTRAL NERVOUS SYSTEM IRRITATION FOLLOWING INJECTION OF TESTICLE EXTRACTS. M. C. Winternitz and (by invitation) E. Mylon and R. Katzenstein, New Haven, Conn.

Abstract. Extracts of testicle, particularly, but those of kidney also, when injected into animals result in symptoms of irritation of the central nervous system. These may be associated with change in the coagulation time of the blood and can be suppressed by preliminary injection of small amounts of the same extract and by heparinization. Fractionation of testicle extract results in a nucleoprotein-lipid complex, the injection of which causes thrombi to be formed, but as the extract is purified the symptoms of irritation of the central nervous system become less.

THE ISOLATION OF "POLIOMYELITIC" STREPTOCOCCI FROM THE STOOL IN ACUTE EPIDEMIC POLIOMYELITIS. Edward C. Rosenow, Rochester, Minn.

Abstract. Serial dilutions were made at steps of 1:10,000 in rapid succession, alternately in tall tubes of dextrose-brain broth and soft dextrose-brain agar, of 10 per cent emulsions of stools. Streptococci always grew in low dilutions in mixture with *Bacillus coli* or other bacteria, irrespective of the source of the stools, but streptococci grew in high dilutions, usually in pure culture, most often when stools were obtained from patients during the acute stage of epidemic poliomyelitis, and less often during convalescence; only occasionally when stools were obtained from persons suffering from non-epidemic disease; and almost never when stools were obtained from well persons remote from cases of poliomyelitis. Streptococci isolated in high dilutions from stools of persons ill with poliomyelitis had characteristic virulence and specific agglutinating and precipitating properties. "Virus" takes were repeatedly obtained in mice with emulsions and filtrates of stools from patients ill with acute poliomyelitis if cultures from the stools yielded the "poliomyelitic" streptococcus.

Fourteen per cent of 229 mice inoculated intracerebrally with filtrates or intranasally with emulsions of stools from 46 cases of active poliomyelitis, 2 per cent of 55 mice inoculated with emulsions of the stools of 8 patients convalescing from poliomyelitis, and 1 per cent of 136 mice inoculated with emulsions of the stools of 41 well or ill persons remote from poliomyelitis died in 3 to 21 days from causes other than pneumonia. Death in "virus time" occurred in 21 per cent of 187 mice that received by serial passage brain emulsions, or filtrates of brain emulsions, of mice that died late after inoculation of filtrates or emulsions of stools from 14 cases of active poliomyelitis. Lesions in many of the mice that died late resembled those of encephalopolyomyelitis.

STUDIES OF NEUTROPHILIC MATURITY FOLLOWING INJECTION OF FRACTIONS OF STERILE EXUDATES (RABBIT). George H. Reifstein (by invitation), Syracuse, N. Y.

Abstract. Sterile exudates were produced, following the methods of de Haan, Mudd and co-workers, and others, by the intraperitoneal injection of 0.9 per cent sodium chloride solution into rabbits. The supernatant fluid fractions of such exudates were injected intravenously into 11 other rabbits and leukocyte studies made. Previous studies (*Am. J. Path.*, 1941, 17, 219) have shown that neutrophilic leukocytoses regularly follow several hours after such injection. In the present experiments, hourly determinations were made of the maturity of polymorphonuclear neutrophilic leukocytes in the circulating blood of these rabbits. Neutrophilic cells were classified in various groups of maturity according to the appearance of their nuclei.

Preliminary studies showed that the neutrophilic maturity of these and other rabbits studied without injection remained relatively constant during a 6 hour daily period. Following an intravenous injection of 5 cc. of supernatant fluid fraction of a sterile exudate, neutrophilic "left shifts" were constantly observed in a series of 14 experiments. Following repeated injections of 5 cc. of supernatant fluid fractions into the same rabbits, these "left shifts" were considerably greater and more progressive. Similar studies of



By vote of the Council of the American Association of Pathologists and Bacteriologists this issue of *The American Journal of Pathology* honors H. GIDEON WELLS. The senior authors of the articles have all been pupils of Dr. Wells.

magnanimity, as Chief of the Laboratory, never took advantage of their arduous and brilliant work.

Of the three books written by Dr. Wells—one of which was translated into German and French—the most important, which passed through five editions (the last in 1925), is his monumental volume of 790 pages on "Chemical Pathology." A pioneer in its field, this comprehensive book was as original and as important as was Claude Bernard's "Physiologie Générale." The other two volumes are special extensions of chemical pathology as it pertains to tuberculosis and immunity. In this field Dr. Wells was "bahnbrechend," not only for his encyclopedic collection of available data but for his own biochemical contributions. This work constitutes more than any other his monument to lasting fame.

In his modest way, Sir Charles Bell boasted that in his lifetime he had, as teacher of anatomy, neurology and surgery, taught some 700 to 800 medical students. Considering the times this certainly constituted a huge number since pupils then chose their instructor rather than being assigned to a teacher by circumstance. Even so, I know of no distinguished pupils of Charles Bell, such as Wells's Harry Corper, Maud Slye, Esmond Long and Paul R. Cannon. All are productive scholars and teachers. I have no doubt that had Dr. Wells taught in the times of Bell, his students would have equalled, if not exceeded, in number those of his predecessor of a century before; for of all the teachers I have had Dr. Wells was the best, with the late Dr. Julius Stieglitz a close second. Perhaps I can sum up the matter best by altering an old Latin adage: "Magister seu poeta nascitur; orator fit."

Wells, a born teacher, was never an orator but his good humor in the classroom and his "wise-cracks" or "gags" helped much to keep the classes on the alert. I happened to be in his first autopsy course together with Harry Corper, Robert L. Benson, Clyde Brooks and Esmond Long. Well's unique manner of quizzing instead of lecturing was most effective in inculcating into us and into thousands of other students the rudiments of pathology. It took a master in pedagogy to use this method effectively. Others have tried it but few have achieved more than mediocre success.

At this happy moment when his colleagues in his own field welcome this special number of the *American Journal of Pathology* in honor of Dr. H. Gideon Wells, his more intimate associates rejoice in having known personally this fine investigator, superb teacher, generous executive and appreciative friend.

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TRIBUTE TO DR. H. GIDEON WELLS,
INVESTIGATOR, SCHOLAR, TEACHER (MAGISTER)

ARNO B. LUCKHARDT, M.D.

(From the Department of Physiology, University of Chicago, Chicago, Ill.)

When it was suggested that I write a few words about Dr. Wells, my teacher and colleague, diffidence nearly deterred me from accepting this rare privilege for even under the most favorable conditions I would feel unequal to this otherwise happy assignment. On the other hand, honorable recognition of distinguished *living* contributors to human welfare is, on the whole, unfortunately so rare that any mite must be offered which will foster a custom which should be observed more commonly. In attempting this, there looms up one obvious difficulty in that the resulting effort may appear too much like an obituary notice or an "In Memoriam." No such difficulty can arise in the present instance, however, for Dr. Wells is still much alive and active, and though retired from active duty at the University, still evinces that exuberance of spirit and that sense of humor which it was my good fortune to discover, appreciate and profit by when I first met him 36 years ago.

The detailed academic "Vita" of Dr. Wells can be found by the interested reader in any copy of "American Men of Science." More to the point is the printed bibliography of his published writings which lies before me, comprising twelve typewritten pages of titles of articles of diverse nature and published in a variety of journals. These began in 1897 and have continued down to 1940; and they range from the expected Virchowian pathology to the biochemical aspects of physiologic and pathologic processes and "Education in Hospital and Laboratory." As time went on, the emphasis was more and more on the biochemistry of disease and immune reactions, particularly as occurring in calcification and tuberculosis. Malignant neoplasms had always attracted the attention of Dr. Wells, and since Maud Slye became associated with him, his interest has centered in that direction for more than 2 decades. As director of this work from the beginning and as able counselor to Miss Slye and Miss Holmes, Dr. Wells with great

the meaning of keratinization in carcinoma of the bronchus and the relationship to its ability to spread. Until such an interpretation is forthcoming its clinical significance must remain vague. It seemed to us, therefore, that any correlation between a particular microscopic picture and the extent, and therefore the operability, of the lesion is so often hazardous that various morphological classifications based on the microscopic picture of the lesion are all of but little clinical value.

Recently we had reason to modify the foregoing conceptions somewhat.² As our experience with early lesions increased we began to encounter a type of tumor that could be set apart from the more classical type of bronchiogenic carcinoma. It was associated with a very definite clinical picture. It was seen in females just as often, if not oftener, than in males. It usually occurred in younger people and often was characterized by rather long-standing clinical features, many of the patients giving symptoms dating back a number of years before more active growth had occurred. The pathologic picture was different from the usual type of carcinoma in the sense that the lesion protruded into the bronchus as a nodule covered with epithelium. This epithelium was generally squamous and overlay an area of fibrous tissue distal to which tumor cells were seen. These tumor cells were often arranged in cords and alveoli resembling unaërated fetal lung tissue. Not infrequently there also was found evidence of mesodermal tissue such as cartilage, bone, muscle and often an extreme vascularity. When the specimen along with the entire lung or lobe was removed surgically it was noted that the tumor was often collar-button-shaped. The larger portion of it was frequently outside the lumen of the bronchus extending into the lung parenchyma. The fact that only a minor portion was evident and the major portion invisible suggested the analogy to an iceberg. It may or may not have metastasized. Almost universally there was some type of congenital abnormality of the lung tissue. This was present most frequently as an abnormal number of lobes. Other pulmonary malformations were observed. Because of the resemblance to fetal lung tissue, its association with congenital abnormalities of the lung and the mixed tissue elements present, it occurred to us that this lesion was probably one intimately connected with congenital malformation of the lung.

EPITHELIAL METAPLASIA IN CONGENITAL CYSTIC DISEASE OF THE LUNG *

ITS POSSIBLE RELATION TO CARCINOMA OF THE BRONCHUS

NATHAN A. WOMACK, M.D., AND EVARTS A. GRAHAM, M.D.

*(From the Department of Surgery, Washington University School of Medicine,
and Barnes Hospital, St. Louis, Mo.)*

As primary cancer of the lung is becoming more and more frequently an operable lesion, opportunities to observe earlier growths are likewise becoming more frequent. This is of greatest importance for it affords a chance to correlate the microscopic picture, as determined from bronchoscopic biopsy, with the gross pathological findings in the early stages of the disease. In such a way a confident understanding of the clinical picture may be reached. Before this understanding can be brought about, however, such a correlation between the microscopic and clinical picture must be established.

As was suggested several years ago,¹ forms of classification based on the microscopic picture as seen in far advanced lesions have but little to offer the clinician. Many of the tumors, by the time they have become widespread, exhibit a marked degree of pleomorphism producing different phases of cellular differentiation depending upon the site from which the section was taken. However, this does not always occur, for at times widespread tumor growths will be seen in which sections taken from many areas will show the same phase of cellular configuration.

There seems to be but little correlation between the amount of cellular differentiation and the extent of the growth. Squamous cell cancer of the bronchus offers a good example of this. It is not unusual to find a very well differentiated squamous type of tissue in an early lesion; nor is it unusual to find a similarly well differentiated type of squamous epithelium in a far advanced lesion. The same lack of correlation between the amount of cellular differentiation and extent of growth holds true in squamous cell cancer that shows even poor differentiation. We have on numerous occasions seen relatively poorly differentiated squamous epithelium in lesions definitely operable. In view of the fact that normal bronchial epithelium under certain conditions possesses the ability to form keratin, it is difficult to interpret

* Received for publication April 2, 1941.

distention and distortion. After fixation for 24 hours the specimen was sectioned along the axis of the major bronchi. A longitudinal slab of the entire lung or lobe, as the case might be, approximately 1 cm. thick, was then made and this was cut into smaller blocks which were then fixed in formaldehyde for 24 hours and sectioned after paraffin embedding. The sections were stained with hematoxylin and eosin, phosphotungstic acid and hematoxylin, and, in instances where indicated, with Mallory's aniline blue and orange G stain. The sections were studied particularly in relation to the normal architecture of the bronchi and bronchioles with attention to the relative amount and type of tissue going into the formation of these structures.

Of the 9 specimens submitted to such study, in 3 there was evidence of epithelial overgrowth found microscopically which upon subsequent examination of the gross specimen was not apparent with the naked eye. In none of the specimens was any gross evidence of tumor formation seen. It must be assumed, therefore, that the structures to be described are entirely local in their growth.

REPORTS OF CASES

Case 1

A. V., male, age 34. Five years before admission to the hospital the patient had suffered an attack of bilateral bronchopneumonia. He was in bed 1½ months and spent an additional 2 months in convalescence. Two years before admission to the hospital he developed a cough which became progressively worse and was productive of white, foul sputum, particularly in the morning. There was a gradual development of dyspnea. Occasionally the sputum was blood-tinged and occasionally there was slight pain in the left chest. Upon admission to the hospital he was found to have numerous cavities throughout the entire left lung which upon bronchoscopic examination were found to be filled with pus. There was no evidence of bronchial obstruction nor of tumor formation. The right lung was normal. Accordingly, on April 3, 1939, the entire left lung was removed by Dr. Brian Blades.

Gross Pathology. Figure 1 shows the gross appearance of the lung. The visceral pleura was covered by firm, fibrous adhesions. Palpation of the lung revealed atelectasis in the lower lobe with many spotty, cystic areas more noticeable in the upper lobe. On sectioning the lung, the pleura was found to be thickened to the extent of about 4 mm. The bronchi and bronchioles, particularly of the upper lobe, extended to large cavities lined by

This conception then would recognize two main types of bronchial tumors. One of these arises in apparently adult epithelium, is much more frequent in the male after middle age, usually possesses some tendency on the part of the epithelium to form keratin and generally spreads fairly rapidly. The other type probably arises in tissue of embryonic type, is generally associated with congenital pulmonary disorganization, does not favor either sex and may remain in the same site for many years before invading other tissue. Each of these types, therefore, presents features both morphologic and clinical that make it distinguishable in most instances. Such a classification has been of the greatest value to us from the standpoint of both therapy and prognosis. The latter type includes the tumor which in the literature is commonly designated as bronchial adenoma. In our opinion this tumor should be regarded as potentially malignant, although in many instances it may show no tendency to invade other tissues for many years and doubtless occasionally has been successfully removed through the bronchoscope.

We have been particularly interested in those tumors often associated with developmental defects in the lung.

If such a conception is true, in all probability one should occasionally be able to find evidence of epithelial overgrowth in pulmonary tissues which are the site of congenital malformation. The following report is concerned with efforts to identify such evidence of overgrowth in the epithelial elements of disorganized pulmonary tissue before such overgrowth was evident clinically.

OBSERVATIONS

Because of the availability of surgical material, congenital cystic disease of the lung was taken as a lesion evidencing abnormal pulmonary formation. All of this material presented severe secondary infection with concomitant distortion of the anatomical picture. Accordingly, only those cases were used in which adequate, careful studies could be made. There were 9 such patients, 6 of whom were treated by total pneumonectomy and 3 by lobectomy.

Immediately after the surgical removal of the lung or lobe the specimen was distended by the injection of Kaiserling's solution into the patent bronchi, care being used not to produce over-

more profuse in the morning. During these 2 years he had lost approximately 15 lbs. Upon admission to the hospital he was found to have numerous saccular dilatations involving the entire left lung, which upon bronchoscopic examination were found for the most part to be filled with pus. The right lung was relatively normal. There was no evidence of bronchial obstruction of any sort. On April 27, 1939, the entire left lung was removed (E. A. G.).

Gross Pathology. The material examined consisted of the entire left lung. There were many adhesions involving the pleura which were unusually firm. On cut section the bronchi were seen to be tremendously dilated and extended into large cystic cavities which were filled with pus. The lingula was the only portion of the upper lobe involved in the cystic process. The lower lobe was markedly involved and in many areas presented atelectasis.

Microscopic Pathology. Microscopic study showed the bronchi to be markedly dilated. The lining epithelium was everywhere intact and in some places changed to a squamous type. The bronchi in many places were dilated and in most areas presented a notable absence of some of the normal mesodermal elements of the bronchial wall, notably cartilage and smooth muscle. In other areas, however, there was an overabundance of smooth muscle. There was very little scarring or fibroplasia of the bronchial wall and a relatively small amount of infiltration by inflammatory cells. These for the most part were lymphocytes and plasma cells. In the lumen of the bronchi, however, there was considerable pus. Upon studying the extension of the bronchi into the periphery of the lung, cystlike structures lined by bronchial epithelium were encountered. The air sacs were often markedly dilated while in other areas considerable atelectasis was present. An interesting feature around some of the bronchial walls was an ingrowth of the epithelium of the air sacs into the wall itself forming small cystlike cavities. In several areas nests of epithelial cells were observed in the bronchial wall just beneath the epithelium. For the most part these cells were cuboidal, although here and there they showed a tendency to become spindle-shaped, apparently growing at random (Figs. 3 and 4). There was no encapsulation. The nuclei for the most part were vesicular and relatively symmetrical. There was only a scant cytoplasm in many of the cells, which was vesicular. Mitotic figures were not apparent. The size of the cystic cavities, the

walls of varying thickness depending upon the amount of inflammation present. Within these cysts there was found a great deal of pus. The lower lobe for the most part was atelectatic although here and there one could see fairly large cysts.

Microscopic Pathology. Very little lung parenchyma was encountered. The interalveolar septa were markedly thickened, presumably as the result of chronic inflammatory change. The alveoli all seemed to be considerably dilated. The cystic cavities were lined by bronchial epithelium, some of which was ciliated and beneath which one found a chronic inflammatory reaction, nonspecific in type, with complete absence of many other bronchial structures. Very seldom was cartilage found. Smooth muscle for the most part was absent. There were no mucous glands. Whether these structures were never formed or whether they had been destroyed by inflammation cannot be determined; the latter would be most unusual. In one area there were noted masses of epithelial cells tending to be squamous in type which had delimited external borders and which were normally differentiated and showed no evidence of mitotic figures. Figure 2 is taken through this area. These cells had their origin well away from bronchial tissue. Although the cell masses were not encapsulated, they gave the appearance of very slow growth.

In the larger bronchi the structures were for the most part normal. Sections through the lymph nodes showed only chronic inflammatory change. The picture throughout all of these sections was that of a congenital lesion of the lung in which the bronchi and bronchioles were enormously dilated into cyst formation. Because of the size of the cysts and because they were lined by perfectly normal bronchial epithelium and showed rather minimal inflammatory change as compared to an unquestioned case of bronchiectasis, one is justified in calling this congenital cystic disease of the lung rather than a marked bronchiectatic change.

Case 2

L. F., male, age 48. Fifteen years before admission to the hospital the patient developed a productive cough which persisted for 2 years and which came on spontaneously. There was gradual improvement. About 2 years before admission to the hospital the patient developed influenza and following his recovery there was a return of the cough, which has remained constant and productive. The sputum was purulent and foul and was much

was thickened and there was a suggestion of a change to a squamous type. On the whole, however, there was a small amount of inflammatory exudate to be found in these dilated bronchi. There was almost complete absence of cartilage throughout the smaller bronchi and bronchioles. In several areas there was marked metaplasia of epithelial elements. These areas were often found in regions normally occupied by mucous glands and presented as large islands of cellular material, fairly well differentiated and showing only a few mitotic figures (Figs. 5 and 6). There was a striking tendency for growth along the subintimal portion of the pulmonary vein. Often the epithelium would project into the vein, giving the appearance of a thrombus of tumor tissue. In most instances, however, it was possible to see that the endothelium was intact overlying the metaplastic tissue. Adjacent to these areas of epithelial hyperplasia there could also be seen areas of new muscle formation without any appreciable relation to the normal architecture. This muscle was of the smooth type and was not associated with any bronchus. Examination of the lymph nodes showed chiefly sinus hyperplasia with epithelial elements. Other portions of the lung showed various phases of atelectasis and pneumonitis such as one would expect from the gross picture. Because of the obvious evidence of malformation presented in this lobe it was felt that the inflammatory process here was probably secondary and that the nature of the lesion was one of congenital cystic disease.

DISCUSSION

It is possible that objections might be raised to the consideration of these pathological processes as congenital cystic disease rather than as bronchiectasis. We have classified these lesions as congenital cystic disease because the lungs have shown evidence of developmental malformation. The fact that clinical symptoms in these three individuals did not develop until relatively late is of no significance. Frequently, clinical symptoms in congenital cystic disease of the lung do not occur until the development of an infectious process. Where cysts are not infected it is not unusual to find them symptomless.

The epithelial changes described in the three cases were not apparent to the naked eye. The blocks were examined for such

absence of normal bronchial elements in the wall and the relatively slight amount of inflammatory reaction present in the wall all suggest that the lesion present here is one of congenital cystic disease rather than that resulting from an acquired infection.

Case 3

W. T., male, age 39. At 3 years of age the patient had a suppurative lymphadenitis and osteomyelitis which healed in approximately 3 years. At 9 years of age he developed pneumonia, following which he had a productive cough with foul sputum which had since been constant. There had been no hemoptysis nor had there been any great change in his condition. One year before his admission to the hospital the patient developed influenza, at which time signs suggestive of pulmonary tuberculosis were found and he was sent to a tuberculosis sanitarium. Here he was thought to have bronchiectasis of the right lower lobe and was referred to the Barnes Hospital where he was found to have large saccular dilatations chiefly confined to the right lower lobe. On bronchoscopic examination these cavities were found to be filled with pus. There was no evidence of obstruction to the bronchi nor was there any evidence of pulmonary tuberculosis. Accordingly, on August 27, 1940, the right lower lobe was removed by Dr. Brian Blades. Because no evidence of an interlobar fissure was seen at the time of operation it was necessary to make a resection of that portion rather than to perform an ordinary lobectomy.

Gross Pathology. Examination of the gross specimen showed the entire surface to be covered by irregular patches of fibrous tissue representing adhesions which had been divided at the time of operation. Landmarks on the specimen were rather difficult to identify. The posterior inferior portion of the lobe was dense and of a firmer consistency than the anterior portion of the specimen. On cut section it was found that many of the larger bronchi were markedly dilated. The walls of these bronchi were considerably thickened. The entire posterior portion of the lobe was almost completely consolidated and the anterior portion fairly well aerated. In the posterior portion near the base there was a large cyst measuring 1.5 by 2.5 cm.

Microscopic Pathology. The features of chief microscopical interest were limited to the bronchi. The markedly dilated walls showed considerable irregularity, there being many infoldings with the production of an irregular lumen. The epithelium was intact over a good portion of the bronchi. However, in certain areas the mucosa was lost, leaving a connective tissue base. In other areas the columnar epithelium lining the dilated bronchi

DESCRIPTION OF PLATES

PLATE 107

FIG. 1. Sagittal section through entire lung. The lower lobe presents numerous large cysts, the walls are moderately fibrosed and there is very little normal lung tissue present.

FIG. 2. An area of metaplastic epithelium in which the cells are for the most part spindle-shaped and the margins of the cellular areas are often clearly demarcated by palisading of the nuclei. There is no evidence of encapsulation. $\times 250$.

changes before sectioning. In no instance was there any evidence of extension to contiguous structures such as the mediastinum, nor was there any evidence of distant metastasis. These lesions were found only after careful search, requiring in some instances as many as fifteen separate blocks of tissue. While the process described obviously represents abnormal cellular growth, because of its local situation we have not felt justified in considering it malignant from a clinical standpoint.

While the changes mentioned do not resemble the picture seen in the so-called mixed tumor of the bronchus that we have previously described, we feel that they do represent a similar process; namely, a disturbance in the fundamental structural tissue growth so often seen in areas of abnormal tissue organization. Where this situation is encountered in other parts of the body it is not unusual to find malignant manifestations following environmental stresses and strains. Whether such abnormal epithelial proliferations as described here are concerned in carcinomatous change is a question that we shall consider in a subsequent publication.

CONCLUSIONS

Studies were undertaken to determine the presence of abnormal epithelial overgrowth in congenital malformation of the lung. In 3 of 9 patients operated upon for congenital cystic disease of the lung, evidence was found of such overgrowth which consisted for the most part of masses of poorly differentiated epithelial cells tending to appear as spindle cells or cuboidal cells showing a definite tendency toward invasion but nowhere presenting any evidence of metastasis. In none of these specimens was the lesion apparent to the naked eye.

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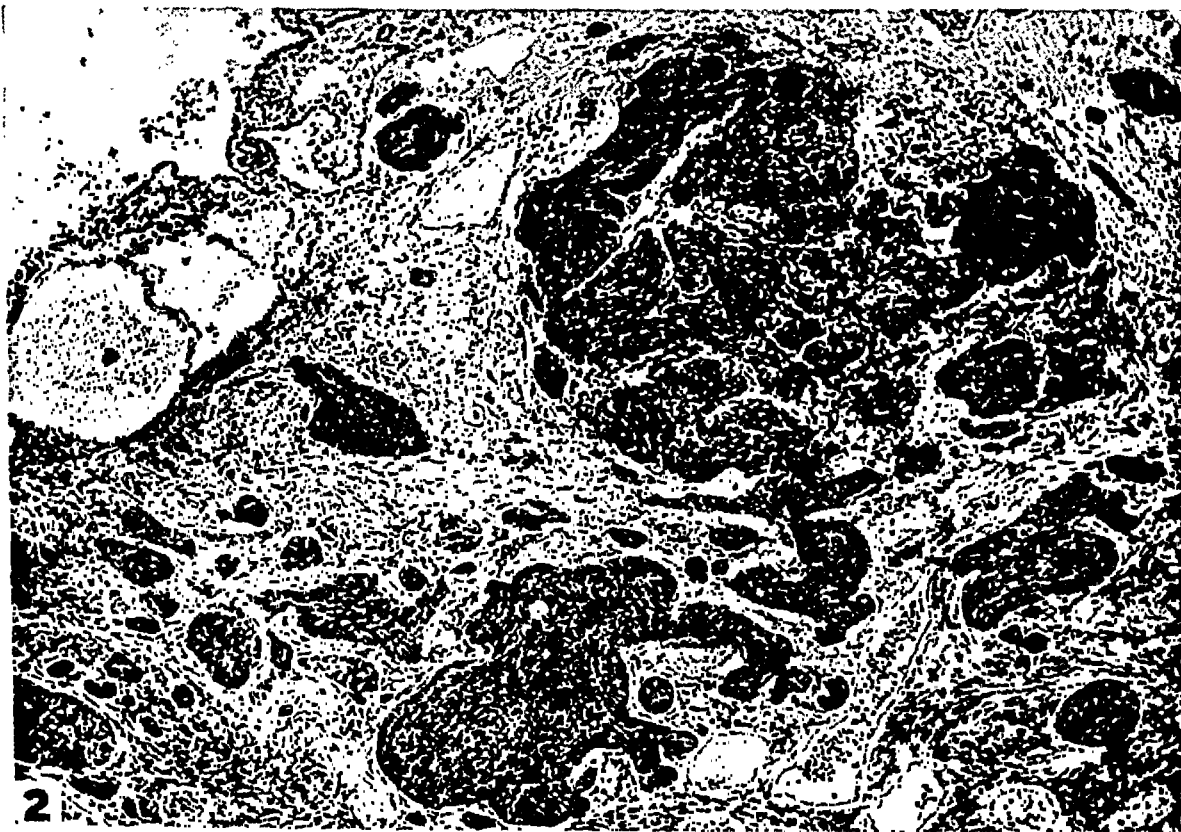
PLATE 108

FIG. 3. A low power view of epithelial overgrowth showing relationship to the bronchial epithelium. There is a moderate round cell reaction throughout the wall of the bronchus. No cartilage is visible. The epithelium lining those air sacs adjacent to the bronchus is of cuboidal type.
× 300.

FIG. 4. Another area similar to that described in Figure 3 is shown. The epithelial masses give the appearance of having invaded lymph vessels.
× 600.



1

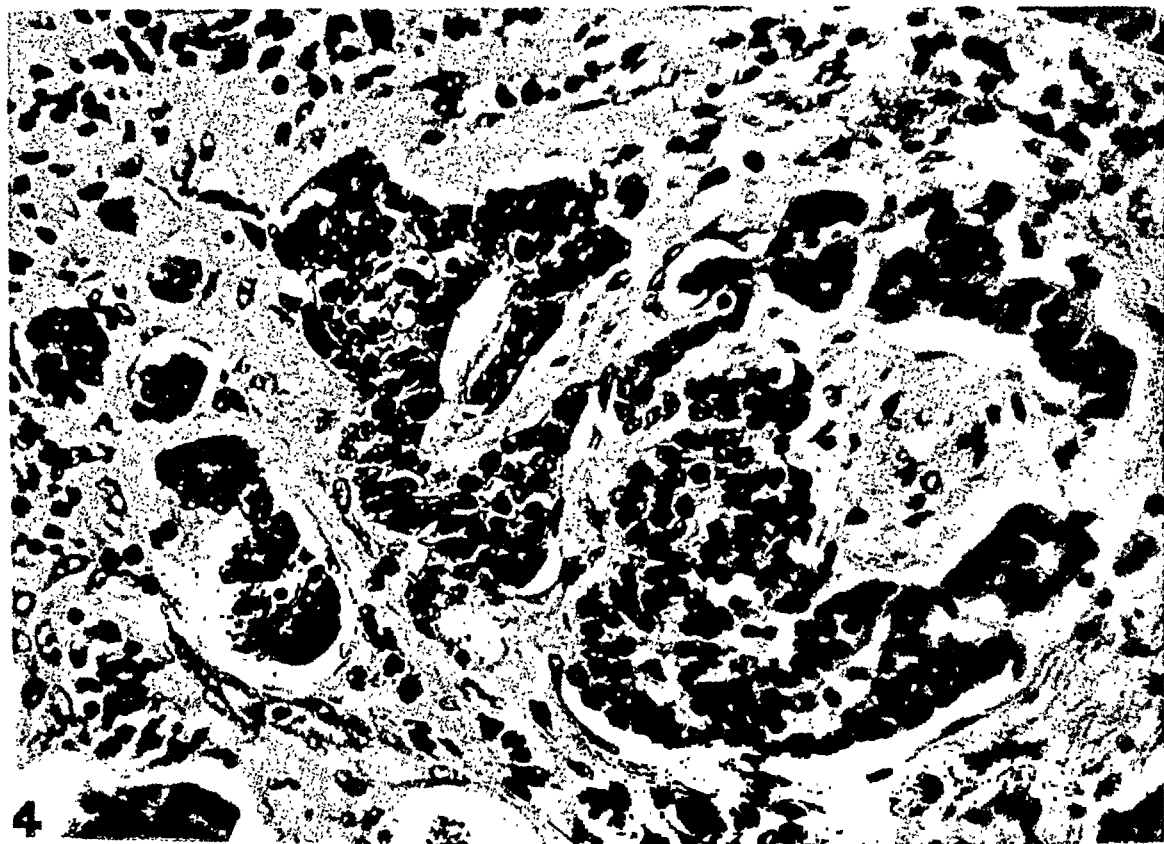
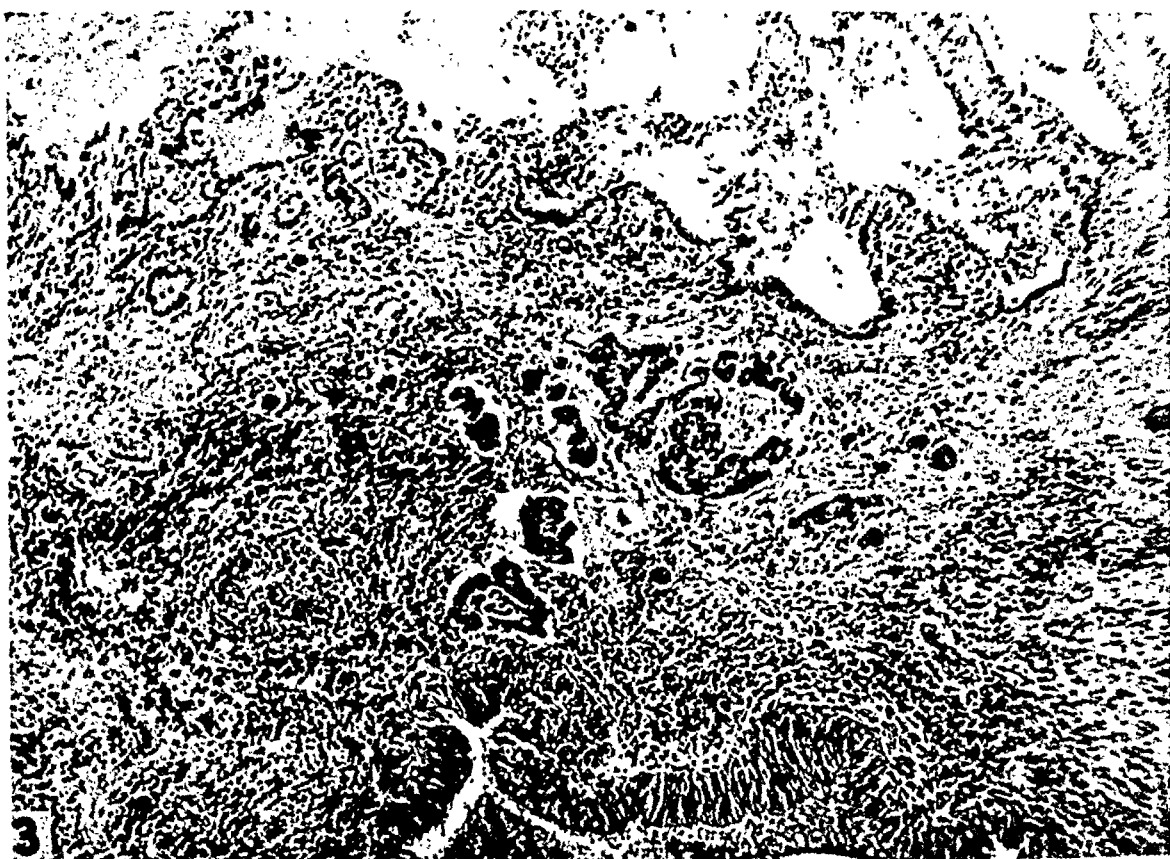


2

PLATE 109

FIG. 5. Low power view of an area of epithelial hyperplasia found well away from any bronchial wall. There are numerous small cavities and a large amount of smooth muscle is found in the stroma. These masses of cells are growing without any evidence of encapsulation. $\times 250$.

FIG. 6. Groups of epithelial masses segregated in a dense area of smooth muscle and fibroblasts. The cells tend to be spindle-shaped, although the margins of the masses evidence palisading of the nuclei. $\times 250$.



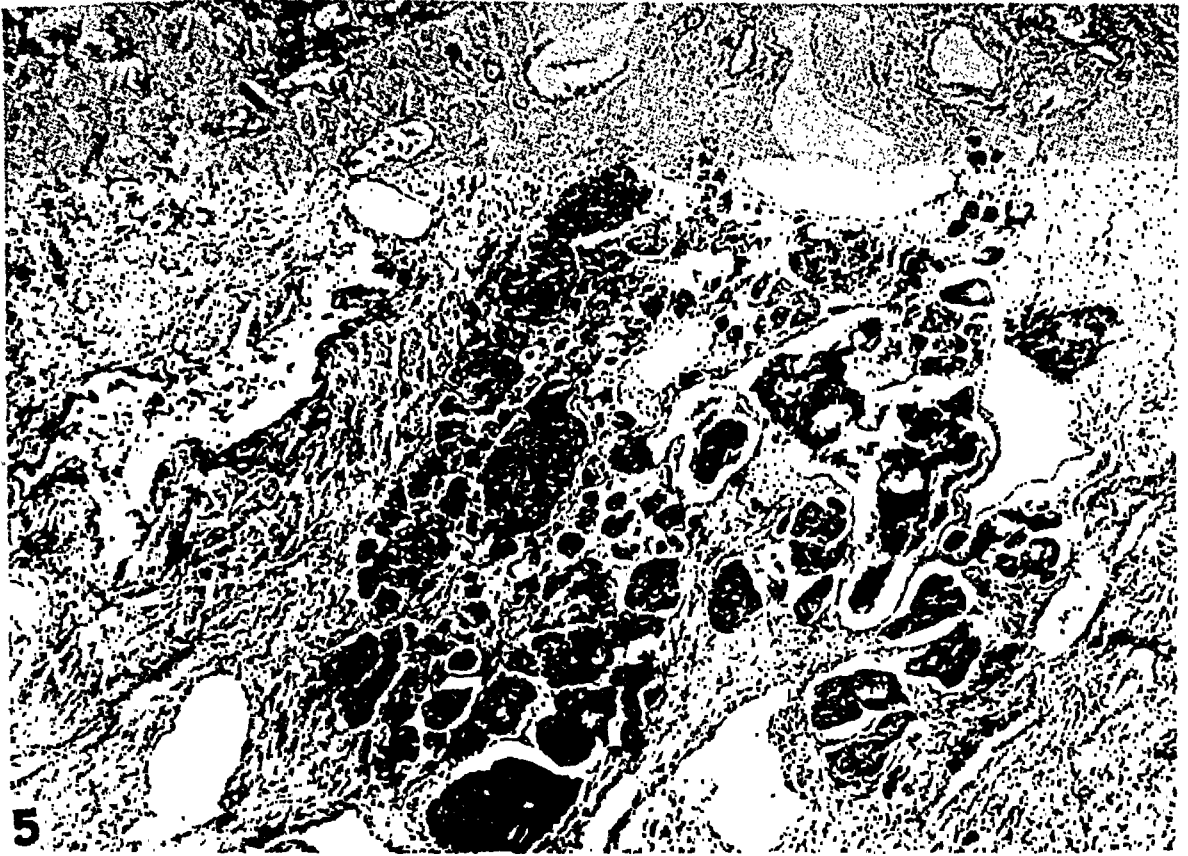


Table II shows series 2 and the ages and causes of death of the parents of the strains of origin 3 and 7, and of their two hybrid crosses.

TABLE I
Totals of Strains 85 and WQ and Their Reciprocal Crosses

Series 1		Total offspring
WQ	10 inbred generations from	♀ 369
	♀ (carcinoma, mammary gland, 15 mo.) X	♂ 395
	♂ (chronic nephritis, 17 mo.)	764
85	8 inbred generations from	♀ 423
	♀ (pulmonary infection, 22.8 mo.) X	♂ 502
	♂ (unknown infection, 6.4 mo.)	925
Reciprocal crosses:		
85/WQ	8 inbred generations from	♀ 1133
	♀ (intestinal infection, 11.7 mo.) F ₄ of 85 X	♂ 1244
	♂ (carcinoma, lung, 16.5 mo.) F ₄ of WQ	2377
WQ/85 I	8 inbred generations from	♀ 309
	♀ (carcinoma, lung, 16.4 mo.) F ₄ of WQ X	♂ 322
	♂ (multiple liver adenomas, 24.5 mo.) F ₄ of 85	631
WQ/85 II	6 inbred generations from	♀ 286
	♀ (retroperitoneal hemorrhage, 6 mo.) F ₄ of WQ X	♂ 318
	♂ (chronic nephritis, 19 mo.) F ₄ of 85	604
WQ/85 III	4 inbred generations from	♀ 122
	♀ (carcinoma, mammary gland, 12 mo.) F ₄ of WQ X	♂ 121
	♂ (same as parent ♂ of II)	243

TABLE II
Totals of Strains 3 and 7 and Two of Their Hybrid Crosses

Series 2		Total offspring
Line 3	4 generations from	♀ 125
	♀ (nephritis, 18.9 mo.) X	♂ 111
	♂ (nephritis, 19 mo.)	236
Line 7	9 generations from	♀ 402
	♀ (carcinoma, mammary gland, 14.4 mo.) X	♂ 365
	♂ (pneumonia, 10.9 mo.)	767
Note: the strain was formed by a back-cross of the F ₁ ♀ with the parent ♂		
Hybrid crosses:		
Line $\frac{3}{7}$	2 generations from	♀ 15
	F ₂ ♀ of line 3 (hypertrophic heart, 19.6 mo.) X	♂ 5
	F ₄ ♂ of line 7 (nephritis, 5.5 mo.)	20
Line $\frac{7}{3/7}$	7 generations from	♀ 369
	F ₂ ♀ of line 7 (intestinal infection, 12.5 mo.) X	♂ 396
	F ₂ ♂ of line $\frac{3}{7}$ (sarcoma, body wall and subcutaneous tissues, 25.7 mo.)	765

HEREDITY AS DETERMINING THE TYPE AND SITE OF CANCER AND THE AGE AT WHICH IT OCCURS *

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I wish to present, tersely, evidence for the genetic influence in the occurrence of malignancy. Evidence will be advanced for the genetic control of: (1) the type of malignancy—carcinoma, sarcoma and leukemoid diseases; (2) the site of malignancy—breast, lung, body wall and subcutaneous tissues; (3) the age at which malignancy will occur.

The figures used are derived from the study of four strains of mice with their hybrid crosses. Tables I and II describe the material used in these studies. The total number of mice involved in the two series is 7332.

The parents selected from strain WQ for the reciprocal crosses shown in Table I were 3 litter sisters and their litter brother, derived from four inbred generations of breast carcinoma; that is, the mother, grandmother, great-grandmother and great-great-grandmother had each died of carcinoma of the breast.

Of the 3 litter sisters, ♀ I died of lung carcinoma at 16 months without breast carcinoma; ♀ II of hemorrhage at 6 months, too early to be tested for malignancy; ♀ III at 12 months of breast carcinoma. The litter brother died at 16.5 months of lung carcinoma. Therefore in strains 85/WQ and WQ/85 I, there are not only reciprocal crosses between the two strains of origin, but also reciprocal tests of lung carcinoma.

The parents chosen from strain 85 for the reciprocal crosses were 1 female and her 2 litter brothers, whose parents and grandparents died of intestinal infection without malignancy.¹ The same male was used in crosses II and III. The female died at 11.7 months of intestinal infection, too early to be tested for malignancy, but her genetic behavior was closely like that of her litter brother I who died of multiple malignant liver adenomas at 24.5 months. The litter brother used for crosses II and III died of chronic nephritis at 19 months.

* Received for publication March 26, 1941.

is therefore inadequate for testing the genetic potentiality of any form of malignancy except breast carcinoma, since the mice do not live long enough to show whether or not they are susceptible to malignancy of any other site.

Examining the percentages for types in Table III, let us first consider carcinoma. Strain WQ had 92.3 per cent of carcinoma, most of which was carcinoma of the breast, in 221 tested mice. Strain 85 had 44.5 per cent of carcinoma (a low percentage of which was in the breast) in 236 tested mice. The reciprocal crosses showed 67.2 per cent where the male came from the strain having a high percentage of carcinoma; and 63.4 per cent where the female came from the strain having a high percentage of carcinoma. Both of these figures are nearer to the percentage for strain 85 than to that for strain WQ, but they show that where a high percentage of carcinoma was bred in, a high percentage of carcinoma came out in the resulting strain; and that when a strain having a high percentage of carcinoma was crossed with a strain having a lower percentage of carcinoma, both were effective. The average result, where the number of tested mice is large enough, shows a figure between the high and the lower percentages, and nearer to the lower.

Of sarcoma, WQ had only 13.6 per cent. Only 22 mice, however, lived into the test age for sarcoma. Strain 85 was adequately tested for sarcoma. Of 210 mice living well into the test age, only 9 per cent showed sarcoma. The resulting reciprocal strains both had a low percentage of sarcoma. As the percentages show, many of these mice had more than one type of malignancy. Many showed two types and some all three types developing concurrently.

Of leukemic disease, WQ had 64 per cent among the 50 mice that lived into the test age. Strain 85 had 22.2 per cent among 225 tested mice. The reciprocal hybrid strains showed percentages between those for WQ and 85 and nearer to the lower percentage strain.

Series 2 has been selected because of the contrast it offers to series 1. In this series, strain 3 showed a medium amount of malignancy of each type, and strain 7 showed a high percentage of carcinoma and of sarcoma, 56.5 and 44.9 per cent respectively, and only 17.4 per cent of leukemia.

TABLE III
Percentage Incidence of Types of Malignancy in Series 1 and 2

Series 1	WQ	85	85/WQ	WQ/85 I, II, III	Totals reciprocal crosses
% carcinoma	92.3 (221)*	44.5 (236)	67.2 (944)	63.4 (568)	65.7 (1512)
% sarcoma	13.6 (22)	9.0 (210)	13.0 (690)	10.3 (369)	12.1 (1059)
% leukemia	64.0 (50)	22.2 (225)	25.4 (736)	40.8 (436)	31.1 (1172)
Series 2	3	7	$\frac{3}{7}$	$\frac{7}{3/7}$	
% carcinoma	22.2 (81)	56.5 (418)	0.0 (20)	51.3 (318)	
% sarcoma	17.1 (82)	44.9 (390)	80.0 (20)	60.4 (386)	
% leukemia	20.9 (91)	17.4 (384)	0.0 (20)	21.8 (316)	

*The figures in parentheses represent the number of mice tested in each category.

Table III presents evidence for the genetic control of types of malignancy. The most rigid age test has been used in all of these figures. All noncancerous and cancerous mice dying before 22 months of age have been discarded as not adequately tested. Of course all mice actually showing the type and site of malignancy under consideration have been included, such mice obviously being tested for their type of malignancy at whatever age they died. Thus even very young cancerous mice with the tested type of malignancy are included, but noncancerous mice of the same and even greater ages are excluded as not certainly tested for cancer.

The average ages of the noncancerous mice in the different strains are from 25 to 29 months, and the age span of these mice is from 22 to 45.5 months. Mice in general reach their full development at 3 months or earlier. If 3 months in the life of a mouse is considered the equivalent of 12 years in the life of a human being, 12 months in a mouse would be the equivalent of 48 years, and 24 months would be the equivalent of 96 years of human life.

WQ is a strain in which only 5.6 per cent of the total offspring lived beyond 22 months. Over 78 per cent of the strain died at an average age of 13 months, the females nearly all of breast carcinoma, and the males of nephritis and wounds. The strain

in the totals of this hybrid cross which gave 51.3 per cent of carcinoma, slightly less than the 56.5 per cent of strain 7.

From these strains there were developed (Table IV):

1. Approximately 100 per cent strains of carcinoma and of sarcoma.
2. High percentage strains (about 1 out of 2) for each type.
3. Medium percentage strains (about 1 out of 4) for carcinoma and leukemic disease.
4. Low percentage strains (about 1 out of 8 or more) for sarcoma and leukemic disease.
5. One 0 per cent strain for carcinoma and for leukemia.

These facts demonstrate the genetic control of susceptibility to types of malignancy, irrespective of sites. They show that when sarcoma or leukemia is bred in, sarcoma or leukemia comes out in the resulting lines; that when carcinoma is bred in, carcinoma comes out in the strain; and that when all three types are bred in, it is possible to extract lines some of which show all three

TABLE V
Incidence of Breast Carcinoma and Ovarian Adenoma in Series 1 and 2

Series 1	% mammary gland carcinoma	% ovarian adenoma
WQ	95.1 (203)*	11.1 (36)
85	14.7 (163)	30.3 (165)
Reciprocal crosses:		
85/WQ	38.1 (1 out of 3-) (698)	37.5 (552)
WQ/85	46.5 (1 out of 2+) (387)	32.4 (259)
Total of reciprocal crosses:	41.1 (1085)	35.9 (811)
Series 2		
3	0 (124)	2.1 (48)
7	0 (402)	48.2 (247)
3/7	0 (15)	0 (15)
$\frac{7}{3/7}$	0 (369)	42.0 (169)
Totals:	0 (910)	

* The numbers in parentheses represent the number of tested mice.

Of the hybrid derivatives from these lines, 3/7 was a small strain of only 20 mice, 15 females and 5 males. All of the females and 1 of the males had sarcoma. The 4 males that did not develop any form of malignancy were mice that fought and died of wounds and extreme emaciation. All, however, were age-tested for every form of malignancy. Small as the strain is, and although perhaps it does not show its full malignant potentiality, it is striking that among 20 mice a sarcoma strain of 80 per cent was secured in which all of the malignant mice showed only one type of malignancy. It suggests an extracted line of sarcoma.

That it was primarily a sarcoma strain is shown by the results of its further hybridization. When a male from this strain, with sarcoma, was crossed with a female from strain 7 which was high in both carcinoma and sarcoma, to produce strain $\frac{7}{3/7}$, the first generation hybrids showed no carcinoma but only sarcoma. Carcinoma came out in the later generations. The totals for the hybrid line gave 60.4 per cent of sarcoma, a percentage lying between those of the sarcoma lines of higher and lower percentages. Again the influence of the high carcinoma line is shown

TABLE IV
A Realignment of the Percentages Given in Table III

	Approximately 100%	1 out of 2	1 out of 4	1 out of 8	0%
Carcinoma	92.3 (221)*	67.2 (944) 63.4 (568) 56.5 (418) 51.3 (318) 44.5 (236) <hr/> 60.3 (2484)	22.2 (81)		0 (20)
Sarcoma	80.0 (20)	60.4 (386) 44.9 (390) <hr/> 52.6 (776)		17.1 (82) 13.6 (22) 13.0 (690) 10.3 (369) 9.0 (210) <hr/> 11.9 (1373)	
Leukemic disease		64.0 (50) 40.8 (436) <hr/> 43.2 (486)	25.4 (736) 22.2 (225) 21.8 (316) 20.9 (91) <hr/> 23.7 (1368)	17.4 (384)	0 (20)

* The numbers in parentheses represent the number of tested mice.

TABLE VI
Incidence of Lung Carcinoma in Series 1 and 2

	% lung carcinoma	No. tested
Series 1:		
WQ	No estimate	19
85	7.4	204
Reciprocal lung crosses: 85/WQ and WQ/85 I	24.7	943
Crosses from parents with- out lung carcinoma: WQ/85 II and III	12.4	178
Series 2:		
3	19.8	81
7	32.9	398
$\frac{3}{7}$	0.0	20
$\frac{7}{7}$	31.3	307
$\frac{3}{7}$		

is evidence for a minimum of two recessive factors, one for type and one for site.

The incidence of body wall and subcutaneous spindle cell sarcoma (Table VII) was noticeably different in series 1 and 2. In series 1, the percentages were consistently low in the strains of origin and in the reciprocal crosses. In series 2, the percentages were high.

From the strains here reported there were secured:

1. One approximately 100 per cent strain for breast location, and one for body wall and subcutaneous locations.
2. Strains of high percentage (about 1 out of 2) for breast, ovary and body wall locations.

TABLE VII
Incidence of Body Wall and Subcutaneous Sarcomas in Series 1 and 2

	% sarcoma	No. tested
Series 1:		
WQ	9.5	21
85	6.7	208
Reciprocal crosses:		
85/WQ	10.4	681
WQ/85	7.9	367
Series 2:		
3	17.1	82
7	42.9	387
$\frac{3}{7}$	80.0	20
$\frac{7}{7}$	56.2	377
$\frac{3}{7}$		

types of malignancy, some of which show two types of malignancy, and some of which show one type and only one type.

As evidence for the genetic control of the site of malignancy, I have chosen four tumor sites appearing in these two series: carcinoma of the mammary gland, of the ovary and of the lung, and spindle cell sarcoma of the body wall and subcutaneous tissues.

In series 1, Table V, WQ showed 95.1 per cent of breast carcinoma among 203 age-tested females, and the parent female of the strain had breast carcinoma. Strain 85 showed only 14.7 per cent of breast carcinoma (about 1 out of 7) among 163 age-tested females.

In the reciprocal crosses: 85/WQ, in which the parent female came from the strain with low breast cancer, showed 38.1 per cent of breast cancer in 698 tested females (1 out of 2.6); WQ/85 I, II, III, in which the parent females came from the strain with high breast cancer, showed 46.5 per cent of breast cancer in 387 tested females (1 out of 2.2). There is no evidence here of any extrachromosomal factor, nor any evidence for dominance of breast location. On the contrary, the figures show a closer approximation of the hybrid strains to the strain with a low percentage of cancer of the mammary gland, whether the male or the female is from the strain with a low percentage of breast cancer.

Series 2 shows an interesting sequence of strains from which breast cancer was entirely eliminated, as there was none in strains 3 or 7 or in either of their hybrid derivatives, this series totaling 910 age-tested females. Since breast cancer is the common malignancy reported in mice, this is strong verification of a genetic influence in the occurrence of breast-location for malignancy, and of the possibility of breeding it out of families. It is also evidence for the existence of separate factors for the type of carcinoma and its site incidence, for in series 2 there was a high incidence of carcinoma, but no breast-location of the malignancy.

In the findings for lung carcinoma (Table VI) in series 1, there is no evidence for an extrachromosomal factor, wherein they are in agreement with the findings of other workers. Neither is there evidence for any sort of a dominant, nor for either sort of unit character. Rather, in the figures for lung carcinoma there

long-lived and the short-lived strains were long-lived: 47.5 per cent died from 22 to 45.5 months, and 32.2 per cent died under 18 months.

As to the age-incidence of malignancy, strain WQ had an average age at death of 14 months. Only 1.3 per cent of the mice with malignant neoplasms died after 22 months, whereas 86.5 per cent died at an average age of 13 months. In strain 85, the reverse was true. Nearly 60 per cent of the mice with malignant neoplasms died between 22 to 32.4 months of age and only 21 per cent under 18 months. The average age for malignancy was 22.8 months. In the reciprocal crosses, nearly 50 per cent died between 22 to 38 months of age and 34.1 per cent under 18 months. The average age for malignancy was 22.2 months. The age span of those with malignancy was 3.1 to 38.2 months.

TABLE X
*Comparison of the Age Incidence of Tumors of the Same Type
in the Original and Hybrid Strains*

Series	No. tumors	Average age in months	Age span of malignancy in months	% late	% median	% early
No. mammary gland tumors						
WQ	193	13.2	6.9—21	0	10.4	89.6
85	24	21.1	13.2—28.1	50.0	12.5	37.5
Reciprocal crosses	446	18.2	6.2—32.5	23.6	15.9	60.5
No. lung carcinomas						
WQ	9	16.5	10.4—24	11.1	22.2	66.7
85	15	25.9	17.2—32.4	86.7	0	13.3
Reciprocal crosses	255	22.9	9.3—36.3	59.6	18.8	21.6
No. leukemic disease						
WQ	32	14.5	4.4—23.1	3.1	18.8	78.1
85	50	22.1	11.6—31	52.0	26.0	22.0
Reciprocal crosses	365	22.5	3.1—37.4	57.8	18.6	23.6

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

These same facts held regardless of the type or site of malignancy. In the strains with late malignancy, even breast carcinoma, ordinarily an early tumor, occurred as late as 32 months. In the strains with early malignancy, such usually late tumors as lung and liver malignancy occurred as early as 8 months. The average age for leukemia in the strains with early malignancy

3. No strains of high percentage for lung location, but strains with about 1 out of 4 for lung location.
4. Strains with about 1 out of 8 or more for breast, ovary, lung and body wall locations.
5. Strains with 0 per cent of breast, lung and ovary locations. These are not ratios for dominance.

TABLE VIII

Deaths from All Causes in Entire Strains, Giving the Percentage of Totals Living into Late, Median and Early Age Spans

Series 1	No. mice	% late	% median	% early
WQ	338	5.6	16.0	78.4
85	449	45.0	20.7	34.3
Reciprocal crosses	2143	47.5	20.3	32.2

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

TABLE IX

Average Age of Malignancy in the Original and Hybrid Strains with the Percentage Distribution of the Tumors in Late, Median and Early Age Groups

Series 1	No. cancerous mice	Average age in months	Age span of malignancy in months	% late	% median	% early
WQ	222	14.0	4.4—26.2	1.3	12.2	86.5
85	157	22.8	8.9—32.4	59.9	19.1	21.0
Reciprocal crosses:						
F ₁	26	24.3	12.4—35.3	57.7	11.5	30.8
Totals	1249	22.2	3.1—38.2	49.9	16.0	34.1

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

Table VIII gives a consideration of deaths from all causes in series 1, showing the percentage of distribution of deaths in age periods: late (over 22 months); median (from 19 to 21 months) and early (under 18 months). Table IX gives the percentage of incidence of the cancers in the three age spans.

WQ was a short-lived family: only 5.6 per cent lived to be over 22 months old, and 78.4 per cent died at an average age of 13 months. These figures include death from all causes, non-malignant and malignant. Whatever the cause of death of these mice might be, they died early. Strain 85 was a long-lived strain: 45 per cent lived from 22 to 34 months, and 34.3 per cent died under 18 months, this group including 46.6 per cent of the fighting males. The reciprocals derived from the crosses between the

was 14 months, and in the strains with late malignancy, 22.5 months.

In age-incidence for malignancy in general and in the ages for specific types and sites of malignancy, the hybrids uniformly resemble more closely the long-lived strain. In the age at death from all other causes also, the hybrids more closely resemble the long-lived strain. Thus the tendency for death from all causes to be late within a strain seems dominant over the tendency to die early. The tendency to have malignancy late seems to be dominant over the tendency to have malignancy early.

What is the significance of late and early malignancy and its genetic control? May it mean the hereditary transmission of greater and of less resistance to the causative factors involved in the mutation, whether these causative factors are cancerogenic chemicals, hormones, a virus or any other form of chronic irritant? Or does it mean that genetic constitution determines the timespan within which tissues and organs are capable of normal function with regard to all diseases? May it mean that genetics can control the production of an organism with a high degree of resistance against all disease attacks, malignant or nonmalignant, or an organism which will succumb early to any assault?

In any event the tendency to late malignancy is dominant over the tendency to early malignancy, just as the tendency to live long is dominant over the tendency to die early. The dominance of greater resistance over less resistance is demonstrated, which is consistent with the theory of the recessive nature of cancer susceptibility.

TABLE I
Nonsaponifiable Lipids of Livers from Persons Having Neoplasms

No.	Major diagnosis	Liver weight in gm.	% non-saponifiable lipids	Total non-saponifiable lipids in gm. (calculated)	Fatty changes (histological)	Miscellaneous
H-40-191	Carcinoma, tongue	1200	1.28	15.3	Moderate	Also shows moderate portal cirrhosis
4961	Carcinoma, esophagus	1735	0.44	7.6	Slight	
4968	Carcinoma, esophagus	1700	0.55	9.3	Slight	
5016	Carcinoma, stomach	1600	0.38	6.0	None	
5037	Carcinoma, stomach	2010	0.82	16.5	Slight	Also shows a slight portal cirrhosis
5039	Carcinoma, stomach	1800	2.78	41.2	Slight	
4984	Carcinoma, colon	?	0.63	?	Very slight	
4990	Carcinoma, colon	1200	0.65	8.8	Slight	
4938	Carcinoma, lung	1900	0.69	13.2	Slight	Occasional microscopic metastasis
4948	Carcinoma, lung	1350	0.56	7.6	None	
H-40-186	Carcinoma, lung	2250	0.64	10.3	Slight	
H-40-183	Carcinoma, prostate	2150	1.23	26.5	Slight	
H-40-178	Carcinoma, prostate	1900	0.64	12.2	Slight	Liver also shows cirrhosis; contains 3 cavernous hemangiomas
5022	Carcinoma, urinary bladder	1550	0.51	7.9	Slight	
4958	Carcinoma, breast	910	0.70	6.4	Very slight	
4966	Carcinoma, breast	1640	0.40	6.5	Slight	
4991	Carcinoma, breast	1250	0.81	10.1	Slight	Occasional small metastases
4927	Carcinoma, ovary	1960	1.15	22.5	Slight	
4992	Carcinoma, ovary	1800	0.95	17.2	Severe	Occasional tiny metastases
CCH-4-145	Carcinoma, ovary	1900	0.45	8.5	Slight	
CCH-40-485	Carcinoma, adrenal	2980	0.53	15.8	Slight	Estimated 10% metastases
H-40-192	Retroperitoneal sarcoma	1790	0.83	14.9	Moderate	
4943	Lymphoblastoma	1550	0.57	8.8	Moderate	No infiltration into liver
H-40-193	Lymphosarcoma	1560	2.33	36.2	Slight	
4974	Reticulum cell sarcoma	1070	0.34	3.6	Slight	No infiltration into liver
5012	Reticulum cell sarcoma	1500	0.32	5.2	Very slight	
5048	Reticulum cell sarcoma	1350	0.74	10.0	Slight	Small infiltrations into liver
5014	Monocytic leukemia	2050	0.46	9.5	Very slight	
H-40-231	Hodgkin's disease	1730	0.45	8.2	Slight	Estimated 15-20% leukemia
5013	Meningioma	1450	0.41	6.0	Moderate	
4946	Chromophobe adenoma of the pituitary	1775	0.63	11.1	Very slight	Slight periportal infiltration
4980	Carcinoma, branchial cyst	1500	0.33	4.9	Very slight	
H-40-180	Carcinoma, parotid	1230	0.55	6.7	Moderate	Also shows advanced cirrhosis
Average		1665	0.75	12.3		

THE NONSAPONIFIABLE LIPID FRACTION OF LIVERS FROM CANCEROUS AND NONCANCEROUS PERSONS *

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In a recent communication¹ the induction of spindle cell sarcomas in mice by the subcutaneous injection of the nonsaponifiable lipid fraction of livers from persons having cancer was described. The purpose of this paper is to describe such extracts in greater detail, giving the amounts which human livers contain and attempting to make some preliminary correlations of this fraction with the nature of the major disease and with the morphology of the liver.

The original nonsaponifiable lipid extract which proved to be cancerogenic was prepared from the pooled livers of 8 persons. From 9,420 gm. of fresh liver the yield was 65.6 gm. Since that time individual extractions have been made of livers from 33 persons having various kinds of tumors, the livers themselves containing cancer only to the degree stated in Table I. The livers of 19 adult persons not having cancer were similarly extracted. Most of these individual extracts are now under test in animals for cancer-producing ability.

METHOD OF CHEMICAL EXTRACTION

The livers were ground as soon after autopsy as possible and were preserved in an equal volume of 95 per cent alcohol. They were saponified for about 18 to 24 hours by alcoholic potassium hydroxide on a steam bath under a reflex condenser, water being added in a volume equal to that of the alcohol. The amount of potassium hydroxide used was 10 gm. per 100 gm. of liver tissue. The material was then extracted four times with ethylene dichloride. These combined extracts were dehydrated with anhydrous sodium sulfate, filtered, and then evaporated to dryness at reduced pressure. The residue was resaponified for 4 hours with alcoholic potassium hydroxide, without water. For this saponification 0.5 gm. of potassium hydroxide was used for each 100

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RESULTS

A study of Table I shows that the nonsaponifiable lipid fraction of livers in persons with cancer varied from 0.32 per cent to 2.78 per cent (moist weight). The total nonsaponifiable lipid fraction obtained from human livers weighed between 3.6 and 41.2 gm. The individual variation was enormous. There did not seem to be any correlation between the type of neoplasm and the amount of nonsaponifiable lipids. Neither did there appear to be a significant change in the nonsaponifiable lipids if the primary tumor was located in the region of portal blood drainage.

In Table II the results of similar studies on the livers of persons not having cancer are recorded. These also showed great variation in the amount of nonsaponifiable lipids from case to case, although somewhat less so than in patients with cancer. Although the percentage of nonsaponifiable lipids averaged less for these livers, the total weight of this fraction was just as great as in Table I because the average weight of these livers was greater. This appeared to be true because the average age of these patients was less and therefore there was less brown atrophy of the liver. Also these patients did not show cancer cachexia.

The livers showing cirrhosis were analyzed separately because cirrhosis of the liver may be considered a potentially cancerous or in a sense even a precancerous lesion.² The same great individual variation in the nonsaponifiable lipid fraction was shown as in the other diseases, and although the total amount was greater than was shown in the group without cancer, this is probably not significant. In the cases showing cirrhosis there did not appear to be any relationship between the type or the severity of the cirrhosis and the yield of nonsaponifiable lipids. The results are shown in Table III.

In each of the three tables is given the amount of fatty change visible in the livers by microscopical examination of frozen sections stained by scarlet red, and quantitatively expressed crudely as none, slight, moderate or severe. These fatty changes included both fatty degeneration and fatty infiltration and no attempt was made to distinguish between them. There was no correlation between the amount of fatty change visible on microscopical examination and the amount of nonsaponifiable lipids extracted by this method.

gm. of liver, original weight. Extraction with ethylene dichloride was again carried out for four times and the pooled extracts were evaporated to dryness.

The final residue was a flaky, yellow to orange-brown solid with a pungent, penetrating, disagreeable odor.

As given in Tables I, II and III the total nonsaponifiable lipids for each liver were calculated from the percentage of nonsaponifiable lipids and the known total weight of the organ. This was frequently greater than the amount actually extracted because all of the liver was not available for chemical study in most cases.

TABLE II
Nonsaponifiable Lipids of Livers from Persons Not Having Cancer

No.	Major diagnosis	Liver weight in gm.	% non-saponifiable lipids	Total non-saponifiable lipids in gm. (calculated)	Fatty changes (histological)
4945	Lobar pneumonia	1650	0.50	8.2	None
4964	Lobar pneumonia	2080	0.38	7.9	Slight
4977	Ulcerative colitis, nonspecific	1790	0.41	7.3	None
4985	Ulcerative colitis, nonspecific	2200	0.64	14.0	Severe
4940	Abruptio placenta	2560	0.73	18.7	Moderate
4965	Bacterial endocarditis	2200	0.69	15.2	Slight
4971	Syphilitic aortitis	1570	0.64	10.1	Moderate
4973	Polycystic kidneys	1775	0.37	6.5	Slight
4975	Pneumococcic meningitis	2530	0.71	17.9	Slight
4979	Hypertensive heart disease; uremia	1480	0.26	3.9	Moderate
Cor.-18-5-40	Lye poisoning	1710	0.84	14.4	Slight
Average		1959	0.56	11.3	

TABLE III
Nonsaponifiable Lipids of Cirrhotic Livers

No.	Type of cirrhosis	Stage of cirrhosis (microscopical)	Liver weight in gm.	% non-saponifiable lipids	Total non-saponifiable lipids in gm. (calculated)	Fatty changes (histological)
4951	Portal	Advanced	2400	0.76	18.2	Slight
4960	Biliary	Early	1500	0.72	10.7	Slight
Cor.-20-5-40	Portal	Advanced	1980	0.66	13.0	Severe
Cor.-65-5-40	Portal (?)	Mild, inactive	1415	0.94	13.3	Slight
CCH 40-546	Portal	Advanced	1650	0.33	5.4	Severe
Cor.-37-6-40	Portal	Moderate	1340	0.76	10.2	Slight
Cor.-49-6-40	Portal	Moderate	1340	1.47	19.7	Slight
5063	Cardiac	Early	1250	0.64	8.0	Moderate
Average			1609	0.78	12.3	

It is possible that quantitative or qualitative differences between cancerous and noncancerous persons may be discovered when the nonsaponifiable lipid extracts are fractionated.

SUMMARY

The total nonsaponifiable lipids recovered from the livers of 33 persons with cancer, 11 with various non-neoplastic diseases and 8 with cirrhosis of the liver, show no significant differences in amount. The individual difference is enormous and is not correlated with the type of tumor, with the location of the primary tumor, or with the amount of fatty change (fatty degeneration and fatty infiltration) visible in microscopical sections.

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compartment may cause a difference in composition of the new deposit on each stone. The authors and Pepinsky¹ have reported cases in which there was an increasing tendency for the deposition of calcium and pigment on such stones proceeding from ampulla to fundus of the gallbladder. This is in line with the observation reported in the same article that there is an increasing tendency for the deposition of calcium and pigment on stones formed in the presence of increasing degree of obstruction of the cystic duct, either from stone or from inflammation.

Further observations have been made on the subject. Aronson² has reported that much of the dark discoloration of some gallstones is due to a substance which does not give the tests for bile pigments but, judging from stoichiometric analysis, probably consists of polymers containing pyrrole derivatives which are products of degradation of the bile pigments. While in some cases the more recent deposits on the stones contain increasingly more calcium and are increasingly darker in color from ampulla to fundus, in others no calcium is present but they are increasingly darker from ampulla to fundus. These differences in composition occur in varying degrees of intensity in a considerable percentage of the cases in which a chain of large gallstones fills the greater portion of the gallbladder, and the more nearly it is filled the more frequent the occurrence. Some of the features of this variation in composition of stones formed simultaneously are illustrated in the following cases.

REPORTS OF CASES

Case 1

A man, 54 years old, died of abdominal sarcomatosis after an illness of 6 months characterized by weight loss and attacks of abdominal pain. One month before death an oval, firm mass was palpable in the gallbladder region and a roentgenogram of that region revealed two large ring-shaped, radio-opaque shadows, the distal shadow being more opaque than the proximal (Fig. 1). Attempted cholecystography resulted in nonvisualization of the gallbladder by the dye.

At autopsy the *gallbladder* measured about 12 cm. in length and was filled with a solid mass of stones. Its wall was thickened and there was sarcomatous implantation on much of its serous coat. On opening it there were found four large articulated stones filling nearly all of the lumen and thirteen small faceted stones

VARIATION IN THE COMPOSITION OF GALLSTONES SIMULTANEOUSLY FORMED IN THE GALLBLADDER*

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During a period of simultaneous formation of gallstones there is usually considerable freedom of movement of the stones within the gallbladder, and the bile in which they are bathed at any given time is usually of about the same chemical composition throughout the gallbladder. Under these conditions the building material laid down at any one time is of about the same composition in one stone as in another and since the physical conditions of deposition and crystallization are likewise similar throughout, the stones also tend to be similar in form and size. However, with variation in the composition of the bile, in the freedom of its flow and in the pathological state of the gallbladder wall during the period of stone formation, there may be equally great variation in the composition of the different layers of each of the similar stones. This accounts also in large measure for the marked difference in aggregations or crops of gallstones formed in different individuals and even in the same individual at different times. Also any deposits which may be laid down on preëxisting single or multiple stones during a period of formation of an aggregation of stones are usually of the same general composition as the newly formed stones.

Cholesterol stones are frequently seen in small aggregations ranging up to six or seven in number, in which case they grow to be of large size. Up to a certain point they remain mobile, are all bathed in approximately the same biliary content and are very similar in composition. But on exceeding a certain size, they form a chain within the gallbladder, become faceted where their ends come in contact, and partially obstruct the lumen. This obstruction results in stagnation of the bile which increases in degree in each compartment proceeding from the ampullar region toward the fundus. If there is further growth of the stones, the difference in composition of the bile and in degree of stagnation in each

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sponding portions of the various stones. Calcium and phosphorus were present in the central portion of only the fourth or fundal stone and there only as a trace, while in the peripheral portion they were present in the second, third and fourth stones in increasing amounts away from the ampulla and in a small fundal stone they were present in greatest percentages. Bile pigment, the quantitative determination of which was the least reliable, was present in relatively small percentages in all of the stones and

TABLE I
Case 1

	Stone No. 1		Stone No. 2		Stone No. 3		Stone No. 4		Stone No. 5
	C*	P†	C	P	C	P	C	P	
Ether extraction (weight)	89.4%	64.6%	71.1%	72.9%	82.9%	83.7%	87.4%	63.8%	61.6%
Pure cholesterol (colorimeter)	65.2%	48.8%	34.3%	36.6%	56.2%	54.4%	79.4%	48.2%	46.8%
Calcium	o	o	o	trace	o	0.8%	trace	2.9%	4.4%
Phosphorus	o	o	o	trace	o	0.9%	trace	0.8%	2.8%
Pigment	0.1%	trace	0.2%	trace	0.1%	0.2%	0.2%	2.8%	0.5%
Dark residue	0.32%				1.89%				

* Center

† Periphery

there was no constant difference in distribution between central and peripheral portions although the highest percentage was present in the peripheral portion of the large fundal stone. Cholesterol was present in the peripheral portions in higher percentages than in the central portion except in stone No. 2 and there was no constant variation in cholesterol content of the stones progressing from ampulla to fundus.

The dark residue was determined on two samples; namely, one-half of stones Nos. 1 and 2 and one-half of stones Nos. 3 and 4 and one of the small stones. It was found to comprise by weight 0.32 per cent of the first sample and 1.89 per cent of the second sample which corresponds well with the gross appearance of the stones.

Case 2

Case 2 illustrates not only the presence of calcium salts and increased dark coloring matter in the peripheral layers of stones of the distal portion of the gallbladder, but also a large amount

situated about a large stone in the fundus. About 15 cc. of greenish yellow, turbid fluid was present. The cystic duct was patent but the ampulla was blocked and the outlet of the gallbladder was partly obstructed by the large proximal stone (Fig. 2). On inspection it was seen that the large fundal stone was faceted proximally where it contacted the adjacent large stone and on two sides from contact with the smaller stones. The stones were variously colored by bile pigments and in general they were increasingly dark from the ampulla to the fundus. Roentgenograms were made of the intact stones and of slices cut from the center of each large stone (Fig. 3). The proximal stone showed no calcium shadow. The second stone showed lamina of extremely faint calcium density near the periphery. The third stone showed dense calcium shadows on one side and fainter shadows on the opposite side and along the articulated surfaces. The large fundal stone showed a heavy calcium shadow about the entire periphery and a fainter ring of calcium internal to this. The thirteen small fundal stones showed faint central, and heavy circular peripheral, shadows of calcium density, the peripheral layers corresponding in density to those on the large fundal stone. On section the center of each of the four large stones consisted of a radiating, mottled, yellowish stone of cholesterol and a small amount of pigment with extensive cleft formation as shown in Figure 4. On the outside of each stone there were laminated deposits which varied in color with each stone. Those on the proximal ampullar stone were approximately the same color as its central portion but those on the other three stones were darker and the darkness increased in intensity proceeding from ampulla to fundus. The thirteen small fundal stones were dark in color and laminated. Apparently they were of recent origin and grew from the same materials as were laid down on the surface of the large fundal stone.

Samples for *chemical analysis* were taken from both peripheral and central portions of slices of the four large stones and from a ground-up small stone. The results of the analysis are shown in Table I. Since there was variation in appearance of different parts of the central and peripheral portions and since the samples were taken at random from these portions, a considerable degree of variation in chemical composition might be expected in corre-

TABLE II
Case 2

	Stone No. 1		Stone No. 2		Stone No. 3		Stone No. 4		Stone No. 5		Stone No. 6		Stone No. 7†	
	C*	P†	C	P	C	P	C	P	C	P	C	P	C	P
Ether extraction (weight)	42.4%	67.5%	66.2%	70.4%	78.3%	88.2%	72.6%	72.8%	83.6%	74.1%	84.6%	77.3%	84.5%	71.9%
Pure cholesterol (colorimeter)	34.9%	44.4%	34.9%	51.3%	51.2%	55.8%	31.6%	43.6%	40.7%	39.6%	35.0%	48.5%	62.1%	46.5%
Calcium	trace	0.4%	trace	trace	trace	0.5%	trace	1.2%	trace	1.5%	trace	1.9%	0.4%	2.4%
Phosphorus	o	o	o	o	o	1.0%	o	1.1%	trace	trace	trace	1.0%	trace	1.3%
Pigments	o	trace	trace	trace	0.4%	0.3%	0.2%	0.5%	0.3%	1.0%	0.2%	1.1%	0.4%	1.0%
Dark residue	0.8%	0.3%	o	0.5%	0.4%	0.5%	2.5%	2.0%	1.1%	2.2%	1.6%	3.5%	1.0%	5.0%

* Center

† Periphery

‡ Pocketed stone

Case 3

A woman, 69 years old, a patient of Dr. G. M. Crabb, died of carcinoma of the colon.

At necropsy the *gallbladder* was found very large, thickened and filled with three large articulated stones. There were other small dark stones about the surface of the unusually large stone located in the fundus. The exterior of the stones was rough and dark brown, with the intensity of color slightly greater along the surface of the distal portion of the fundal stone (Fig. 9). A drawing of the sectioned stones is shown in Figure 10. The huge fundal stone contained a pure cholesterol stone as its central portion. This was doubtless the primary stone. Secondly, the stones at the centers of the two others were laid down. Then came the laminar deposits on the three stones which were similar in appearance. The peripheral portion of the large distal part of the fundal stone was on the whole the darkest portion. A roentgenogram (Fig. 11) revealed the distribution of the more radio-opaque calcium shadows. There was a thin, irregular, radio-opaque ring about the periphery of the primary cholesterol stone and

of these substances in a stone located in a stagnant pocket off the middle portion.

A female, 50 years of age, had an attack of gallstone colic 1 month before examination. A small mass was palpable in the region of the gallbladder. A roentgenogram, Figure 5, revealed five radio-opaque, circular shadows in the region of the gallbladder, the second of which from the ampullar end was eccentrically located and more dense than the others. Attempted cholecystography resulted in nonvisualization of the gallbladder by the dye. Cholecystectomy was then performed. The gallbladder was thickened and elongated and was almost filled by a chain of stones, one of which protruded in a pocket near the middle portion. A roentgenogram of the unopened gallbladder (Fig. 6) revealed the shadows of seven stones. The two stones at the ampullar end cast practically no calcium shadows. The peripheral portion of the remaining stones cast calcium shadows, that of the pocketed stone being greatest in density.

The *gallbladder* was sectioned from the cystic duct to the fundus (Fig. 7). In addition to the stones it contained about 20 cc. of a cloudy mucoid, orange-colored fluid. The stones were faceted where they came in contact. All were pigmented and the chain increased in depth of color from ampulla to the fundus. However, the darkest stone of all was in the pocket at the middle portion of the chain where stagnation was apparently the greatest (Fig. 8). Section of the stones revealed greater density in the peripheral portion of those casting a calcium shadow in roentgenograms. The centers of all of the stones were similar in appearance, consisting of yellowish brown, radiating material about which there was a narrow, dark pigmented zone. The periphery of each was laminated with variation in color of the laminae. In general the periphery of each stone was increasingly dark in color from the ampulla to the fundus and that of the pocketed stone was darkest of all.

Chemical analyses were made of samples of the central and peripheral portions of slices of each stone (Table II). Again there was some irregularity in the variation but the darker stones toward the fundus and in the pocket contained greater amounts of calcium and phosphorus than the stones in the vicinity of the ampulla, and their peripheral portions contained greater amounts of these than the central portions. Pigment and dark residue were present in greater amounts in the stones that were darker in color.

and formed in a row filling a large part of the lumen of the gallbladder. Growth of the stones beyond that point caused partitioning of the lumen and stagnation of the bile in increasing amounts in each compartment proceeding from ampulla to fundus. With the further growth of the stones in these compartments under conditions of differential stagnation, there resulted a difference in composition of the materials laid down simultaneously on each stone. As stagnation increased from ampulla to fundus, there was an increased tendency for the deposition on the stone of each compartment of salts of calcium and phosphorus, of bile pigment and of a dark material probably representing a degradation product of bile pigment. This differential deposit on the stones showed a tendency to be increasingly dark in color and to cast increasingly radio-opaque shadows in roentgenograms proceeding from ampulla to fundus of the gallbladder. The latter finding is a point of value in diagnostic roentgenology. Stagnation appears to be a factor in the formation of the dark constituent of the gallstones.

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DESCRIPTION OF PLATES

PLATE 110

- FIG. 1. Case 1. Radio-opaque shadows of stones in fundus of gallbladder.
FIG. 2. Case 1. Stones are shown in the opened gallbladder in (a) and after removal in (b).

about the two centers of the more proximately located stones. The periphery of the ampullar stone contained radio-opaque shadows along its articular surface opposite the ductal end. The middle stone also contained calcium shadows along its sides. The shadows of greatest density were in the periphery of the large fundal stone where the calcium salts had been deposited in blotches.

Case 4

In case 4 there was the usual difference in color in stones progressing from ampulla to fundus but calcium was present in extremely small amounts as judged by the roentgenogram.

A woman, 39 years old, had had occasional attacks of biliary colic over a period of 6 years. Physical examination was essentially negative. A roentgenogram revealed absence of radio-opaque shadows in the gallbladder region. Cholecystography showed visualization of the gallbladder which included a large oval, radiolucent mass. Cholecystectomy was performed.

The *gallbladder* showed slight inflammatory change and the cystic duct was patent. Three articulated stones were present which filled the gallbladder and increased in size and in intensity of color from ampulla to fundus (Fig. 12). The small ampullar stone was free of a surface coating but the second and third stones contained lamellar deposits which were brown in color and darkest on the fundal stone. The interior of each was similar to that of the ampullar stone. There were no radio-opaque shadows in the periphery of the fundal stone and only faint lines in its deeper portion indicative of very slight calcium deposition at an earlier stage in the stone formation.

In general these cases indicate that calcium deposition requires a higher grade of obstruction than is necessary for the laying down of the dark deposits. Also stagnation seems to favor the degradation of bile pigments into the dark compounds isolated by Aronson from gallstones. They are usually present in greatest amounts in those portions of stones that were laid down in fields of greatest stagnation.

SUMMARY

Cases are reported in which a small aggregation of similar gallstones, consisting principally of cholesterol, increased in size

PLATE III

FIG. 3. Case 1. Roentgenograms of intact stones in (a) and of slices of four large stones and one intact small stone in (b). Calcium shadows in the two distal large stones and the small stones.

FIG. 4. Case 1. Reproduced from colored drawings of intact and sectioned stones. The peripheral layer of the stones is increasingly dark from ampulla to fundus. The centers of the large stones are of the same composition.

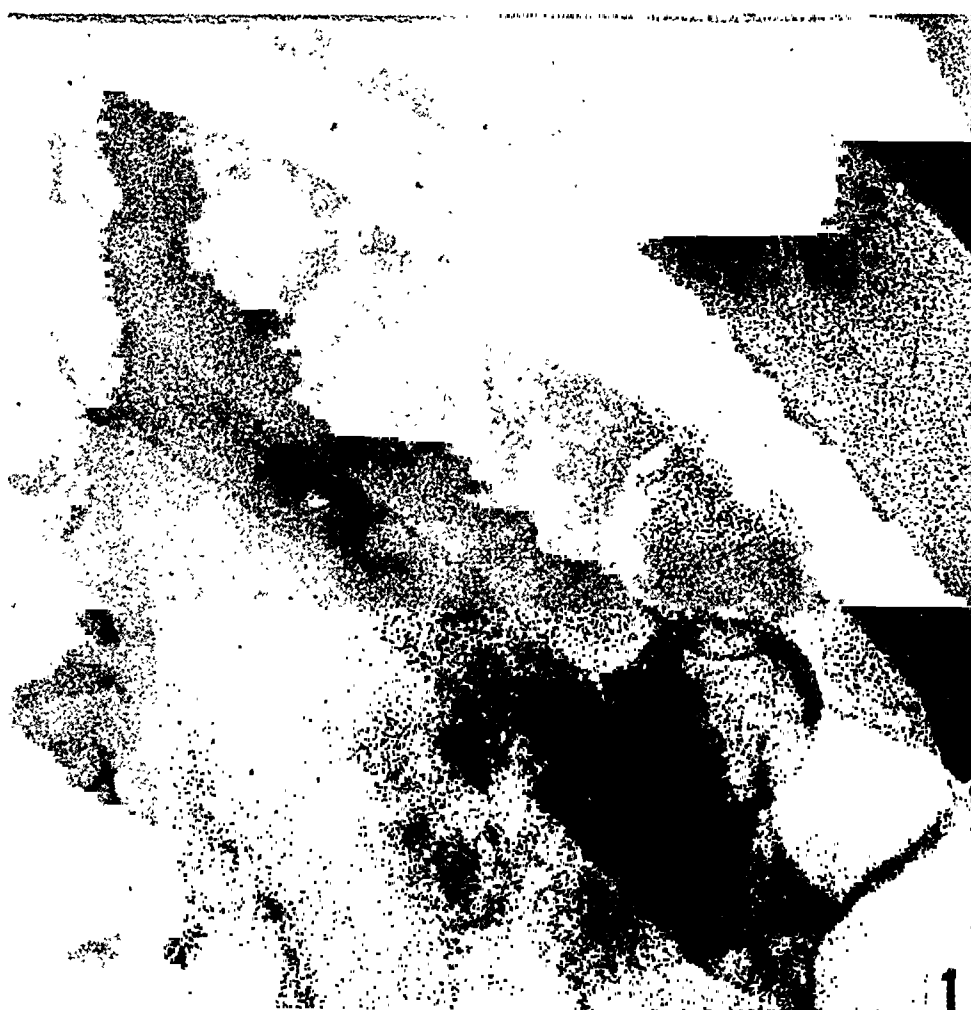
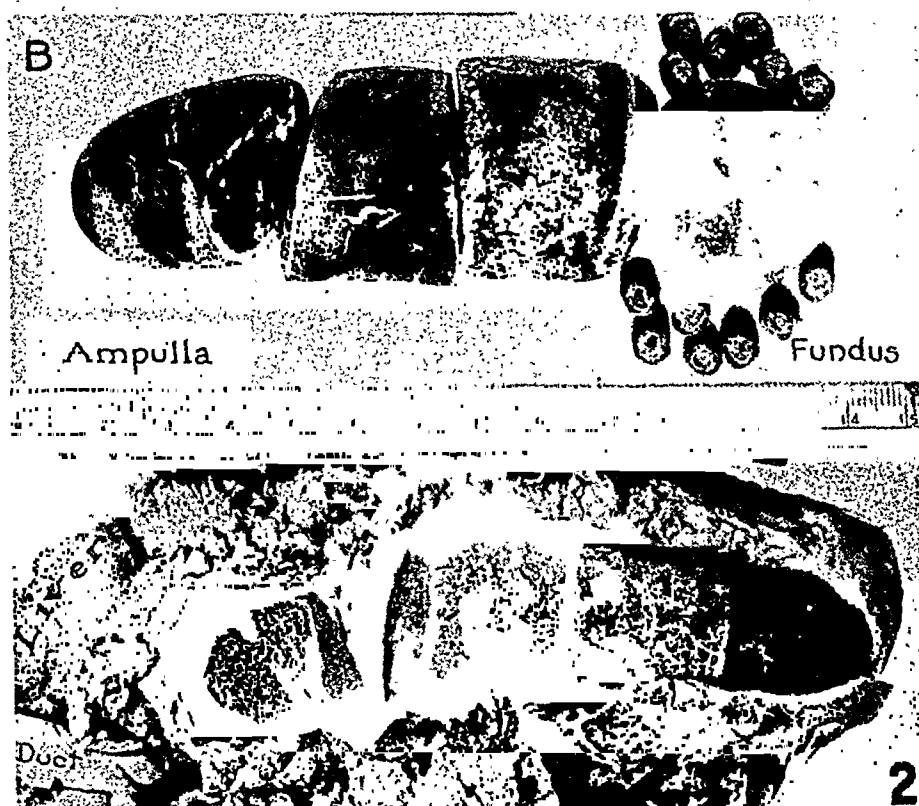


PLATE 112

FIG. 5. Case 2. Ring-shaped radio-opaque shadows of stones in distal half of gallbladder and in pocket.

FIG. 6. Case 2. Roentgenogram of excised gallbladder before opening. There are ring-shaped radio-opaque shadows on the distal four stones, and heaviest on the pocketed stone. The two proximal stones do not show such ring-shaped shadows.

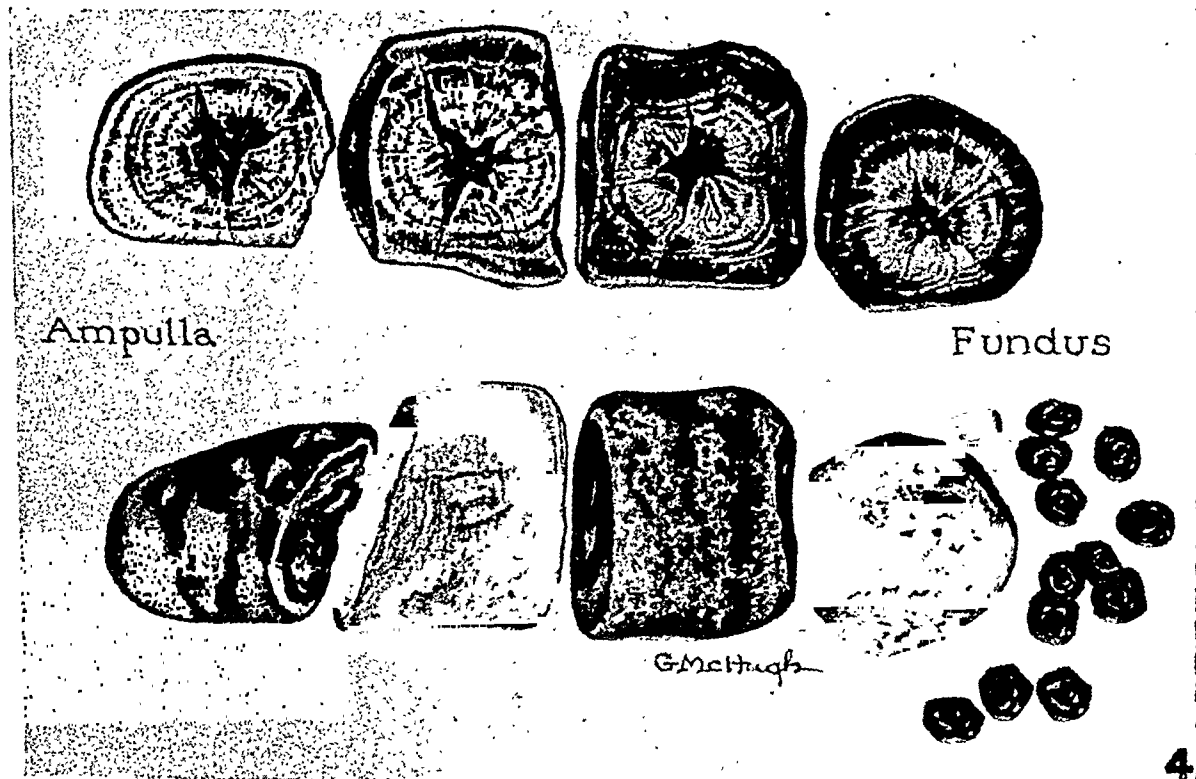
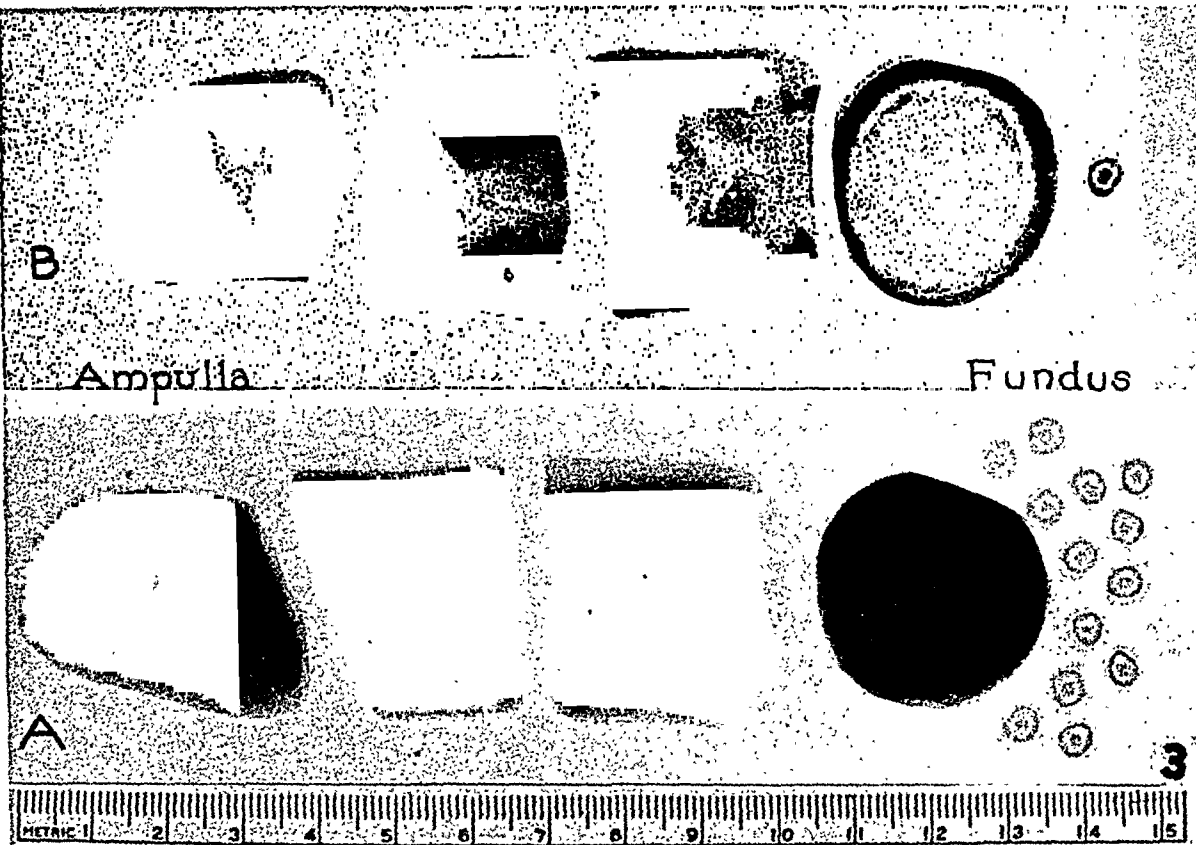


PLATE 113

FIG. 7. Case 2. Incised gallbladder showing the stones in a pocket and in the fundal portion to be darker in color than those of the ampullar region.

FIG. 8. Case 2. Reproduced from colored drawings of exterior and cut section of stones, showing peripheral portions to be darkest in the stagnant fundus and in the pocket.

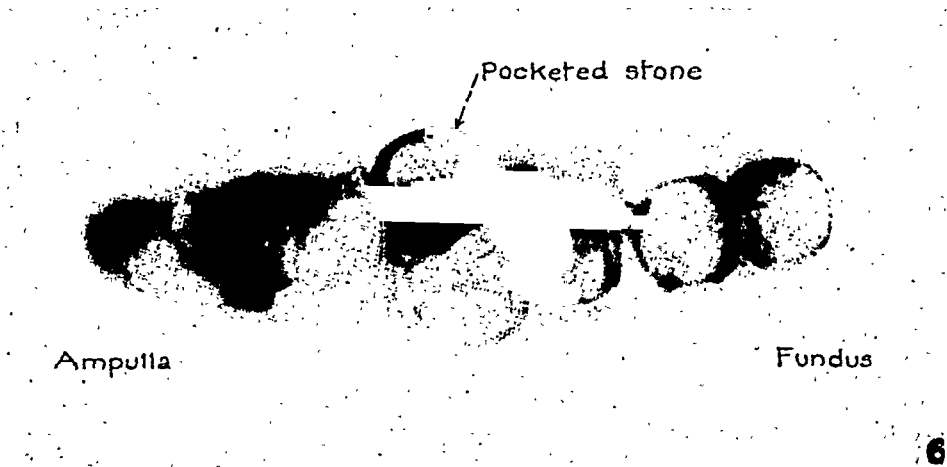
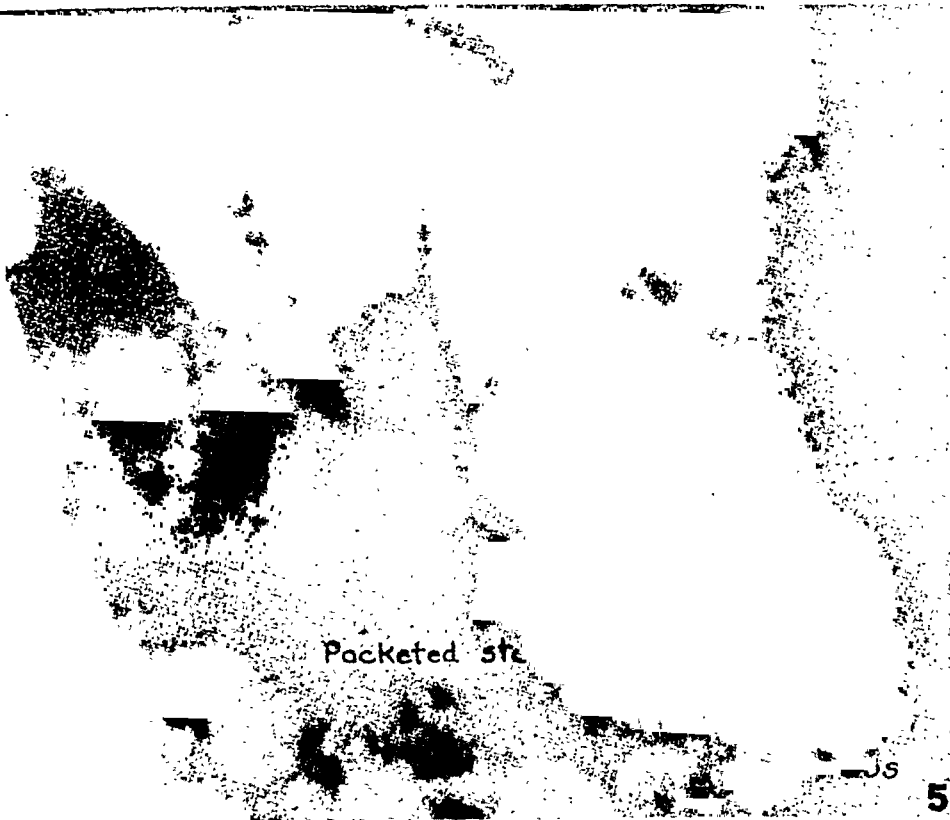


PLATE 114

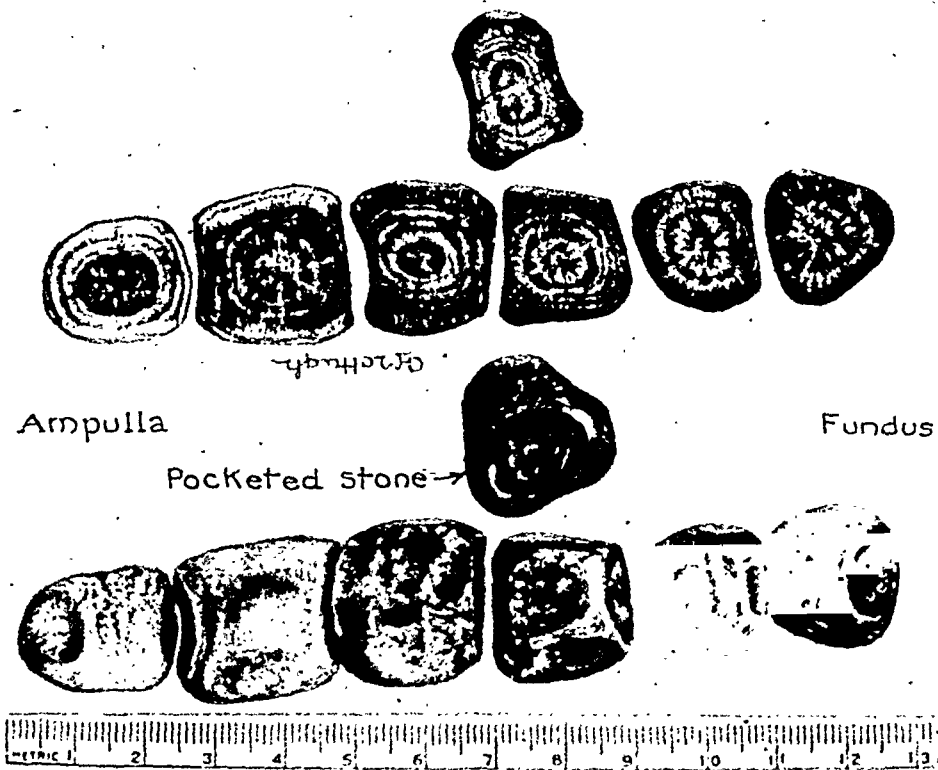
FIG. 9. Case 3. Articulated dark brown stones filling gallbladder.

FIG. 10. Case 3. Reproduced from a colored drawing of sectioned stones. The center of the large fundal stone contains a primary cholesterol stone. The centers of the two proximal stones are formed by cholesterol-pigment stones. Superimposed layers on all three stones are darkest in peripheral portion, and most of all in fundal stone.

FIG. 11. Case 3. Roentgenogram showing calcium shadows on distal end of ampullar stone, on periphery of middle stone and on all sides of large fundal stone. The primary cholesterol stone in the latter shows as a region of the least density.



7

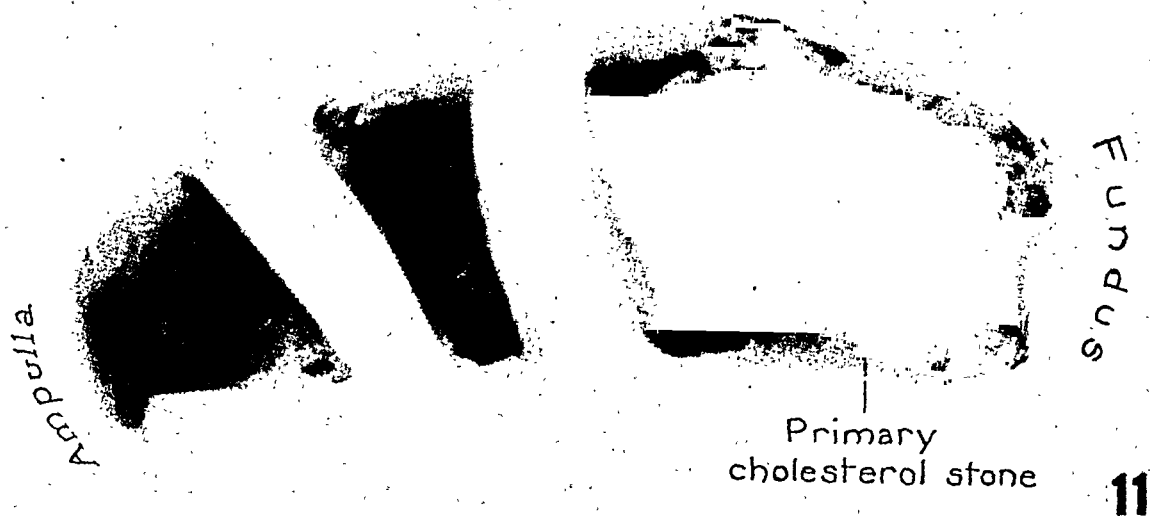
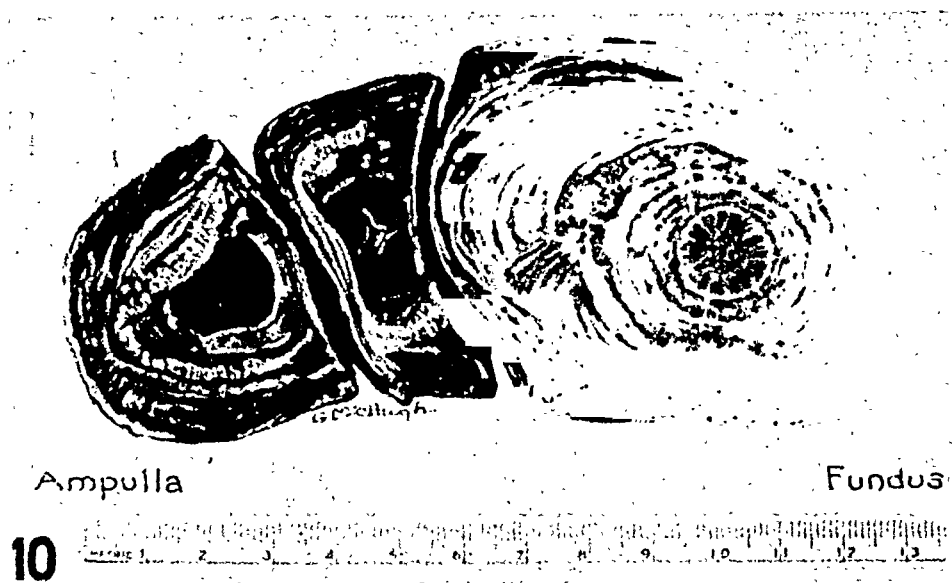


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PLATE 115

FIG. 12. Case 4. Reproduced from a colored drawing. There is an increasingly dark discoloration of the peripheral portion of the stones from ampulla to fundus. Ampullar stone is devoid of laminated peripheral deposit.

FIG. 13. Case 4. The roentgenogram shows absence of a calcium shadow in the periphery of the fundal stone.





Ampulla



12



Ampulla

13

Phemister and Aronson

Composition of Gallstones

study the effect of specific immunity to tuberculosis on a sufficiently extensive group of guinea pigs, and to compare them with a group of nonimmune guinea pigs kept under like conditions and infected with the same suspension of a culture of tubercle bacilli. While this work was being completed, Frappier and Forté⁹ reported at the International Congress for Microbiology in 1939 that they had been able to prolong the life of immune guinea pigs and to prevent infection entirely in some of them.

THE SPECIFIC IMMUNE FEATURE

In order to note any effects of local spread as compared with general conditions in the animal economy, two routes of infection with the highly virulent human tubercle bacilli were chosen: subcutaneous and intravenous (Table I). The number of bacilli injected was also chosen to give the greatest amount of practical information both as to latitude and the time element. It is well known that there is a noticeable effect even with very large injections of virulent tubercle bacilli, since all the pioneering studies of Roemer, Krause, and Calmette were performed in that manner. It is also well known that inexactitudes of the physical factors involved in preparing properly homogeneous infecting materials are to be considered when very small amounts of highly virulent human or bovine tubercle bacilli are used. It is for this reason that complete prevention of infection by specific immunity could not be demonstrated entirely satisfactorily with the highly virulent bacilli used in these experiments and with the highly susceptible guinea pig as the test animal.

With subcutaneous injection (Table I) the immune guinea pigs lived on an average of 216 days while the nonimmune guinea pigs lived on an average of 101 days, both groups having received an infecting dose of 0.0001 mg. of bacilli. The results are even more striking with 0.000001 mg. of bacilli, in which case the immune guinea pigs showed an average duration of life of 319 days as compared with 127 days for the nonimmune guinea pigs. Although there is also a noticeable difference in the arbitrarily graded amount of macroscopic tuberculosis recorded, this alone can hardly present the entire picture of the existing condition. It must be appropriately qualified by statistics on the duration of life and also by the type of tuberculosis found at death. In the

CERTAIN SPECIFIC AND IMMUNOPATHOLOGIC FEATURES OF TUBERCULOSIS *

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In earlier communications ¹⁻³ studies were reported on the behavior of tubercle bacilli within the body. In 1936,⁴ it was found that appropriate previous injections of avirulent human or bovine tubercle bacilli retard the development of subsequent infections with virulent human or bovine tubercle bacilli. Heat-killed avirulent and virulent tubercle bacilli exerted no such effect. Notwithstanding the facts that dissemination of virulent tubercle bacilli from the site of local intracutaneous inoculation is retarded in immune as compared with normal animals (demonstrated by Krause⁵ and verified in this laboratory) and intravenous injection causes a widespread organic dissemination of the virulent bacilli, a definite retardation in the development of the organic disease was noted in immune animals infected intravenously as compared with control normal animals. As a result of quantitative evaluation of the bacilli and the disease,^{6,7} it was found⁸ that the bacillary body sensitized primarily to tuberculo-allergy and served to immunize against virulent infection. The natural filtrate from liquid cultures of tubercle bacilli on a simple synthetic nonprotein medium containing tuberculoprotein, however, sensitizes to anaphylaxis, provokes anaphylactic shock and allergic intoxication, but does not sensitize to allergy nor specifically immunize against virulent infection.

Evaluation of the effects of various procedures upon tuberculosis in the guinea pig has usually been made on the basis of one or both of two criteria: first the comparative extent of tuberculous involvement, giving, however, no information on life expectancy; and second and apparently of lesser value, the duration of life of the infected animals.

Recognizing the inherent difficulties, including the marked susceptibility of guinea pigs to infection by inoculation with highly virulent human tubercle bacilli, an investigation was planned to

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immune guinea pigs a far less progressive type was noted, in so far as could be determined by macroscopic appearance and histologic evidence. As a whole, the more limited chronic forms of pathologic changes were found.

The findings recorded show also a relatively higher pathogenicity of the tubercle bacilli when infection is by the intravenous route of injection. Smaller amounts of bacilli thus given result in earlier death of the animal than is effected by the subcutaneous route. In spite of the general distribution of the bacilli by the intravenous route, these findings corroborate previously recorded results from this laboratory in that there is a striking effect of the specific immunity. This would seem to question the contention of earlier observers who believed immunity was mainly attributed to a local retardation of the spread of the bacilli as a result of the allergic condition. The average duration of life of the immune guinea pigs infected intravenously with 0.00001 mg. of bacilli was 149 days, as compared with 63 days for the nonimmune animals. Those infected intravenously with 0.0000001 mg. of virulent bacilli following immunization lived, on an average, for 240 days, as compared with 114 days for the nonimmune. The amount of anatomic tuberculosis, arbitrarily graded for tabulating purposes, showed also a definite retardation at the time of death in the immune guinea pigs as compared with the nonimmune. Both macroscopically and microscopically there was a more chronic, less progressive type of disease in the immune animals than in the controls, the process being similar to that found after subcutaneous injection. The pathologic characteristics are well shown in Figure 1 from a control guinea pig which died 103 days after virulent infection. In it a more diffuse type of disease was found in all the tuberculous organs, characterized by a more exudative nature, with a predominantly greater number of the younger types of monocyctic cells and numerous granulocytic elements. In the immune animal, which lived 253 days after infection, the tuberculosis was more demarcated in the organs, and the cellular elements in the affected areas were of the more mature monocyctic type, with fibroblastic tissue elements more conspicuous. The gross involvement also showed differences in the majority of cases in favor of the immune animals.

TABLE I

Survival Time of Immune and Nonimmune Guinea Pigs Following Infection with Virulent Human Tubercle Bacilli

Immunized subcutaneously 1 month prior to infection	Tuberculin skin reaction 1:100 Seitz filtrate 2 days before infection	Infection virulent human tubercle bacilli (H 160)*	Survival time after infection in days and amount of tuberculosis (in brackets)	Average survival time after infection in days
1 mg. human avirulent tubercle bacilli	grade 3 in all†	0.0001 mg. subcutaneously	191 (4)† 270 (3) 253 (2) 97 (3) 273 (1)	216
Controls infected only	0 in all		92 (4) 103 (4) 123 (4) 87 (4) 99 (3)	101
1 mg. human avirulent tubercle bacilli	3 in all	0.000001 mg. subcutaneously	375 (4) 370 (4) 339 (3) 221 (3) 292 (2)	319
Controls infected only	0 in all		60 (4) 106 (4) 164 (3) 144 (4) 161 (4)	127
1 mg. human avirulent tubercle bacilli	3 in all	0.00001 mg. intravenously	145 (3) 147 (4) 155 (4) 122 (4) 177 (4)	149
Controls infected only	0 in all		73 (4) 70 (4) 52 (3) 68 (4) 52 (4)	63
1 mg. human avirulent tubercle bacilli	3 in all	0.0000001 mg. intravenously	243 (3) 217 (2) 187 (3) 267 (4) 288 (4)	240
Controls infected only	0 in all		90 (4) 86 (4) 197 (4) 97 (4) 103 (4)	114

* This strain of highly virulent human tubercle bacilli will infect nonimmune guinea pigs in amounts down to about one-billionth of a milligram.

† The skin tuberculin reaction is graded from 0 (no visible reaction beyond that obtained with an equivalent amount of salt solution) to 4 (a reaction 2 cm. in diameter with central necrosis).

‡ The anatomic tuberculous involvement is graded from 0 (no visible macroscopic tuberculosis in any of the organs) to 4 (a generalized marked involvement of all the important internal organs).

have been frequently described¹¹⁻¹⁶ and range from acute edema and cellulitis (granulocytic and monocytic) to profound necrosis of tissue with all types of cellular repair ensuing subsequently, the reaction depending on the hypersensitiveness and amount of tuberculin. However, the organic toxic response in the hypersensitive animal in the absence of local tuberculosis when pure preparations from nonprotein mediums are used merits further consideration, especially in elucidation of the final subject of this study. A series of guinea pigs (Table II) was therefore made allergically hypersensitive. Some of these were then desensitized by appropriately spaced injections of a Seitz filtrate prepared by growing tubercle bacilli on a nonprotein nutrient medium (Wong-Weinzirl). The lungs, livers, spleens and kid-

TABLE II
*The Effect of Tuberculo-filtrate Treatment on General
Tuberculo-allergic Intoxication*

Interval between subcutaneous injection of 1 mg. avirulent human tubercle bacilli and initiation of treatment with tuberculo-filtrate	Route of intoxicating injection* and interval after bacillary injection†	Results
1 month; then 6 weekly intravenous injections of 1 cc. 1:10 filtrate	Intraperitoneally, 70 days	No lethal effect in any of the 5 guinea pigs in this series; a slight toxicity noted
No treatment		All 5 guinea pigs in this series died after 11 to 23 hours
6 weeks; then 5 intravenous injections at intervals of 6 days, 1 cc. 1:10 filtrate	Subcutaneously, 70 days	No lethal effect in any of the 5 guinea pigs in this series; slight toxicity noted
No treatment		Lethal to all 5 guinea pigs in this series within 6 to 20 hours
1 month; then 5 subcutaneous injections at intervals of 6 days, 1 cc. 1:1 filtrate	Subcutaneously, 60 days	No lethal effect in 5 guinea pigs in this series; slight toxicity
No treatment		Lethal within 12 to 36 hours to 4 of 5 guinea pigs in this series; the other guinea pig recovered from a profound toxic reaction

* In all cases, 5 cc. natural filtrate (containing about 4 mg. tuberculo-protein) was used for intoxicating. The intravenous route was not used in order to avoid anaphylactic shock.⁸

† The intoxicating injection of natural filtrate was given 4 to 6 days after the last filtrate treatment (tuberculo-protein).

‡ The same effects were noted in the guinea pigs in which treatment was initiated coincidentally with the bacillary injection. These are omitted from the table.

|| The animals treated intracutaneously showed about the same effects as those treated subcutaneously.

THE SPECIFIC ALLERGIC FEATURE

In a more exact analysis of tuberculosis, the individuality of the specific allergy as distinguished from specific immunity (to infection) must be appreciated, but how far and to what extent specific allergy plays a practical part in the disease has remained a disputed problem. To those who have assumed the normal presence or liberation of tuberculin in the tuberculous organism, the problem would appear to be more easily approached by studies with tuberculin; but to those who have sought more proof than the recovery of tuberculin from cultures or from postmortem materials, the way has not been so direct. Yet the experimental and practical use of tuberculin may throw some light on the problem if applied without carrying conclusions too far and with more exacting adherence to natural conditions. In a recently completed study¹⁰ it was pointed out that, although the specific toxicity in tuberculosis cannot be described definitely as yet because of the lack of information regarding the part played by the products of tubercle bacilli liberated *in vitro* (natural filtrate containing tuberculo-protein—tuberculin) but apparently not liberated in appreciable amounts *in vivo*, desensitization with these products (tuberculo-protein) presents a fascinating problem in tuberculosis, the exact nature and significance of which will have to be disclosed by further experimental investigation. Evidences from the experimental study of tuberculo-anaphylaxis, tuberculo-allergy, and specific tuberculo-immunity would seem to permit question whether the active constituent of *in vitro* natural filtrate from the growth of tubercle bacilli is liberated *in vivo*. In spite of this, desensitization of bacillary tuberculo-allergically sensitized animals, prepared with either avirulent or virulent human tubercle bacilli, can be accomplished by appropriate treatment with natural tuberculo-filtrate (tuberculo-protein—tuberculin) when it is used in relatively small, primarily nontoxic, amounts. Animals thus prepared do not show a local specific skin reaction to natural filtrate (tuberculin) in customary amounts and are likewise protected against a lethal, general, allergic-shock intoxication. Tuberculo-desensitization with primarily nontoxic amounts of natural filtrate (tuberculo-protein) exerts no decided beneficial or detrimental effect upon the course of tuberculosis or upon specific tuberculo-immunity. The characteristics of the local tissue changes produced by tuberculin in the hypersensitive animals

noted did not concern the primary effect of the tuberculo-allergic intoxication but rather those secondary congestive changes consequent upon the general effects of the intoxication, resulting in acute or subacute emphysema of the lungs and circulatory stasis in the other organs. This is borne out by the sequence of events in the following experiments which point to the significance of this feature in the general picture of tuberculosis.

THE COMBINED FEATURES OF SPECIFIC IMMUNITY AND SPECIFIC TUBERCULO-ALLERGY IN TUBERCULOSIS ANALYZED IN A CONCRETE EXPERIMENT

Specific immunity can be demonstrated to exert a definite relative protection against tuberculosis in appropriate experiments, as was shown earlier in this paper and in previous publications. Specific tuberculo-allergy lacks significance in the specific immune effects, but plays a conspicuous rôle in the toxic (allergic) manifestations following the local or general application of the natural tuberculo-filtrate (tuberculoprotein—tuberculin). It may be questioned whether the latter is present at any time as such in the tuberculous economy. Therefore, it appeared desirable to plan an experiment which would more closely approach the conditions occurring naturally in the tuberculous organism and to disclose so far as possible the independence of these phenomena of specific immunity and specific allergy. A number of preliminary experiments showed that when guinea pigs were immunized with avirulent tubercle bacilli followed 1 month later by a large infecting intravenous injection of virulent tubercle bacilli, to imitate the natural mobilization of the bacilli in the tuberculous, the results were rather disastrous. In many cases, the immune guinea pigs suffered an acute or subacute allergic death, while the controls would usually survive for several weeks and then would all succumb within a short interval, to be outlived considerably by all the immune guinea pigs that had not succumbed to the early allergic consequences.

As a result of these preliminary tests and those cited earlier in this paper, the following experiment was performed and has been duplicated since. Twenty-four male guinea pigs of about equal size were divided into three groups of 8 each. Those in group 1 were used as *controls*, and the animals were infected by an intravenous injection of 1 mg. of highly virulent human tubercle bacilli

neys of these animals were examined to note any differences between those dying from tuberculo-allergic intoxication and those protected by desensitization.

Gross examination disclosed distended emphysematous lungs in the animals which had succumbed to the allergic intoxication while the remaining organs appeared to be congested in variable degree, and there was a general congestion of all tissues other than the lungs. The livers particularly showed a distinct lobular pattern characteristic of passive congestion. The guinea pigs that recovered because of a previous protective treatment with filtrate (tuberculoprotein) showed no appreciable congestive reaction of the organs.

Histologic examination of sections of the lungs, livers, spleens and kidneys, stained by hematoxylin and eosin, disclosed the following in the animals that died following the allergic tuberculo-intoxication: The lungs revealed marked thinning of the alveolar walls with occasional apparent rupture, the nuclei stained well and there were no apparent changes in the bronchioli or bronchi. Occasionally the alveolar blood vessels appeared distended in certain areas. The liver sinuses in many of the animals appeared slightly distended and in certain areas the liver cell cytoplasm appeared granular and vacuolar. The nuclei of the liver cells stained normally, and the perilobular vessels as well as the centrilobular vessels were distended. In many cases the spleen revealed a mild distention of the pulp sinuses which were well demarcated. In the kidneys the main changes were found in the malpighian corpuscles in which the tuft vessels appeared to be distended and occasionally the intertubular vessels also were engorged. The nuclei appeared normal and no constant cytoplasmic changes were seen. In summarizing these findings, it should be noted that the predominant changes of the livers, spleens and kidneys of the guinea pigs killed by general tuberculo-allergy were those of congestion, while the lungs showed an acute or subacute emphysema.

For comparison, the organs of guinea pigs dying of acute tuberculo-anaphylactic shock were studied. As might be expected, it was found that the gross appearances in the lungs were much more striking in that distention and alveolar rupture were more common. The congestive mottling of the liver was less striking, with an apparent general engorgement of the entire organ. These studies seemed to indicate that the conspicuous histologic changes

TABLE III
Pathological Findings

Animal and time of death in days	Lungs	Liver	Spleen
Control 11	Slightly distended; congested; tubercles (?). <i>Pneumonitis</i> ; congested; transudate; tubercle bacilli, 2.	Pale; lobules prominent. <i>Fatty infiltration</i> , 4; tubercles, 1; tubercle bacilli, 1.	Measured 4×1.5 cm.; congested; tubercles, 0. <i>Tubercles</i> , 2; congested, 2; tubercle bacilli, 2.
Control 15	Distended; tubercles, 2. <i>Pneumonitis</i> ; transudate; tubercles, 2; tubercle bacilli, 4.	Pale; lobules prominent. <i>Fatty infiltration</i> , 3; tubercles, 2; tubercle bacilli, 1.	Measured 3.5×2 cm.; congested; tubercles, 0. <i>Tubercles</i> , 3; congested, 2; tubercle bacilli, 3.
Control 16	Distended; tubercles, 3. <i>Tubercles</i> , 3, necrotic; transudate; tubercle bacilli, 4.	Pale; lobules prominent. <i>Fatty infiltration</i> , 1; tubercles, 2; tubercle bacilli, 4.	Measured 5×2.5 cm; tubercles(?). <i>Tubercles diffuse</i> ; congested; tubercle bacilli, 4.
Control 16	Distended; tubercles, 4. <i>Pneumonitis</i> ; slightly congested; transudate; tubercle bacilli, 3.	Pale; lobules distinct. <i>Fatty infiltration</i> , 4; tubercles, 2; tubercle bacilli, 1.	Measured 4×2 cm.; tubercles, 2; <i>Tubercles general</i> ; congested; tubercle bacilli, 2.
Control 17	Distended; tubercles, 3. <i>Pneumonitis</i> ; transudate; tubercles, 2; tubercle bacilli, 3.	Pale; lobules prominent. <i>Fatty infiltration</i> , 2; tubercles, 2, necrotic; tubercle bacilli, 2.	Measured 5×2 cm.; tubercles(?). <i>Tubercles diffuse</i> ; congested; tubercle bacilli, 1.
Control 18	Distended; tubercles confluent. <i>Pneumonitis</i> ; tubercles, 2, necrotic; tubercle bacilli, 4.	Pale; lobules prominent. <i>Fatty infiltration</i> , 2; tubercles, 1, necrotic; tubercle bacilli, 3.	Measured 3.5×2 cm.; tubercles, 0. <i>Tubercles diffuse</i> ; congested; tubercle bacilli, 3.
Control 18	Distended; tubercles, 4. <i>Pneumonitis</i> ; transudate; tubercles necrotic; tubercle bacilli, 2.	Brown; lobules distinct. <i>Fatty infiltration</i> , 1; tubercles, 2; tubercle bacilli, 2.	Measured 4×2 cm.; tubercles, 2. <i>Tubercles diffuse, necrotic</i> ; tubercle bacilli, 3.
Control 18	Distended; tubercles, 4. <i>Pneumonitis</i> ; transudate; tubercles necrotic; tubercle bacilli, 4.	Brown; lobules prominent. <i>Fatty infiltration</i> , 1; tubercles, 1, necrotic; tubercle bacilli, 3.	Measured 4×2 cm.; tubercles, 0. <i>Tubercles diffuse, necrotic</i> ; tubercle bacilli, 3.

(H 160) in fine suspension 32 days after initiation of the experiment. Group 2, *specific immune*, was given a subcutaneous injection of 1 mg. of avirulent human tubercle bacilli 32 days prior to infection by the intravenous injection of 1 mg. of virulent human tubercle bacilli (H 160). Group 3, *immune and treated*, was given the subcutaneous injection of 1 mg. of avirulent human tubercle bacilli and treated 7 days later by injecting 1 cc. of 1:10 dilution of natural filtrate, which was repeated every fifth day, a total of 5 injections, until the 27th day, and on the 32nd day after immunization they were infected by being given intravenously 1 mg. of virulent human tubercle bacilli (H 160) in fine suspension. The weight curves of these three groups of animals, the mortality figures and pathologic findings all proved highly enlightening. The mortality figures in days will be presented briefly and the significant findings given.

The duration of life after virulent infection for the *controls* was 11, 15, 16, 16, 17, 18, 18 and 18 days, with an average duration of 16 days; the *specific immune* guinea pigs, on the other hand, lived 19 hours, 36 hours, 8, 9, 17, 19, 22 and 28 days after virulent intravenous infection, with an average life duration of 13 days; and the *specific immune and treated* guinea pigs lived 17, 19, 22, 23, 23, 24, 26 and 27 days, with an average duration of life of 23 days. It is to be noted that the natural filtrate treatment for the specific tuberculo-allergic hypersensitiveness of the specifically immunized guinea pigs prolonged life following the intravenous infection with the virulent human tubercle bacilli from an average of 13 to 23 days by preventing the lethal allergic intoxication usually resulting from such virulent infection (mobilization of bacilli?). The specific immunity prolonged the life of the immune guinea pigs, as compared with the nonimmune controls, from an average of 16 to 23 days.

Pathologic Findings

The gross and microscopic findings in the lungs, livers, spleens and kidneys of these animals will be presented briefly. The organs were sectioned and stained with hematoxylin and eosin for histologic study as well as with carbol fuchsin for noting tubercle bacilli. Aside from the findings recorded (Table III), detailed pathological description yielded little unified significant information.

TABLE III (Continued)

Animal and time of death in days	Lungs	Liver	Spleen
Treated immune 19	Slightly distended; tubercles(?). <i>Pneumonitis</i> , 4; <i>transudate</i> , 1; <i>tubercle bacilli</i> , 4.	Brown; mottled. <i>Fatty infiltration</i> , 2; <i>tubercles necrotic and hyaline</i> , 2; <i>congested</i> , 2; <i>tubercle bacilli</i> , 2.	Measured 5.5×2.5 cm.; brown mottled. <i>Congested</i> , 4; <i>tubercles necrotic and hyaline</i> , 2; <i>tubercle bacilli</i> , 3.
Treated immune 22	Distended; tubercles, 3. <i>Pneumonitis diffuse</i> , 2; <i>tubercles</i> , 2; <i>congested</i> , 2; <i>tubercle bacilli</i> , 3.	Yellow; mottled. <i>Fatty infiltration</i> , 1; <i>tubercles</i> , 2, <i>necrotic</i> ; <i>congested</i> , 2; <i>tubercle bacilli</i> , 2.	Measured 4×2 cm.; brown; diffuse. <i>Congested</i> , 3; <i>tubercles necrotic and hyaline</i> , 3; <i>tubercle bacilli</i> , 3.
Treated immune 23	Distended; tubercles diffuse. <i>Pneumonitis diffuse</i> , 3; <i>tubercles</i> , 1; <i>congested</i> , 1; <i>tubercle bacilli</i> , 4.	Yellow; mottled. <i>Fatty infiltration</i> , 2; <i>tubercles necrotic</i> ; <i>congested</i> , 1; <i>tubercle bacilli</i> , 1.	Measured 4×2 cm.; brown; mottled. <i>Tubercles necrotic and hyaline</i> ; <i>congested</i> , 2; <i>tubercle bacilli</i> , 3.
Treated immune 23	Distended; tubercles diffuse. <i>Pneumonitis diffuse</i> , 2; <i>congested</i> , 2; <i>transudate</i> , 1; <i>tubercle bacilli</i> , 3.	Brown; yellow mottled. <i>Fatty infiltration</i> , 3; <i>tubercles</i> , 2 <i>necrotic</i> ; <i>tubercle bacilli</i> , 1.	Measured 4×2 cm.; brown; diffuse mottling. <i>Tubercles necrotic and hyaline</i> , 3; <i>congested</i> , 3; <i>tubercle bacilli</i> , 3.
Treated immune 24	Distended; tubercles diffuse. <i>Pneumonitis diffuse</i> , 2; <i>tubercles</i> , 2; <i>tubercle bacilli</i> , 4.	Light brown; yellow mottling. <i>Fatty infiltration</i> , 1; <i>tubercles</i> , 3, <i>necrotic and hyaline</i> ; <i>tubercle bacilli</i> , 3.	Measured 5×2.5 cm.; diffuse; necrotic. <i>Tubercles</i> , 3, <i>necrotic and hyaline</i> ; <i>congested</i> , 3; <i>tubercle bacilli</i> , 3.
Treated immune 26	Distended; slight mottling. <i>Pneumonitis diffuse</i> , 3; <i>tubercles necrotic</i> , 2; <i>congestion and hemorrhage</i> , 2; <i>tubercle bacilli</i> , 3.	Light brown; yellow mottling. <i>Fatty infiltration</i> , 1; <i>tubercles necrotic</i> , 2; <i>tubercle bacilli</i> , 2.	Measured 4×2 cm.; dark brown; yellow; necrotic. <i>Tubercles necrotic and hyaline</i> , 2; <i>congested</i> , 2; <i>tubercle bacilli</i> , 3.
Treated immune 27	Distended slightly and mottled. <i>Pneumonitis diffuse</i> , 3; <i>congested</i> , 1; <i>tubercles</i> , 3; <i>hemorrhage and transudate</i> , 2; <i>tubercle bacilli</i> , 4.	Brown; slightly mottled. <i>Fatty infiltration</i> , 0; <i>tubercles necrotic</i> , 2; <i>tubercle bacilli</i> , 1.	Measured 4×2.5 cm.; congested and mottled. <i>Tubercles necrotic and hyaline</i> , 3; <i>congested</i> , 2; <i>tubercle bacilli</i> , 2.

The numerals from 0 to 4 signify the approximate arbitrary grading of the findings. Macroscopic findings are listed in ordinary type; microscopic findings in italics.

IMMUNOPATHOLOGIC FEATURES OF TUBERCULOSIS

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Immune	1	Distended; dark; congested; tubercles, o. Congested, hemorrhage; tubercle bacilli, 1.	Dark; discrete yellow mottling. Congested; tubercles, o; tubercle bacilli, o.	Measured 2×1.5 cm.; congested; tubercles, o. Congested; tubercles, o; tubercle bacilli, o.
Immune	2	Distended; dark; congested; tubercles, o. Pneumonitis; congested; slight hemorrhage; tubercle bacilli, 1.	Brown; irregular mottling. Granulation; tubercles, o; tubercle bacilli, 1.	Measured 2×1.5 cm.; congested; tubercles, o. Congested; tubercles, o; tubercle bacilli, 1.
Immune	8	Distended; tubercles, o. Pneumonitis; congested; transudate; tubercles, o; tubercle bacilli, 2.	Yellow; mottled. Fatty infiltration, 1; tubercles, 1; tubercle bacilli, o.	Measured 3×2 cm.; congested; brown; tubercles, o. Tubercles, 2; tubercle bacilli, 3.
Immune	9	Distended; tubercles(?) . Pneumonitis diffuse; hemorrhage; transudate; tubercle bacilli, 2.	Yellow; mottled. Fatty infiltration, 3; tubercles, 1; tubercle bacilli, 1.	Measured 3×1.5 cm.; tubercles confluent. Congested; tubercles diffuse; tubercle bacilli, 3.
Immune	17	Distended; tubercles confluent. Congested; tubercles, 3; necrotic; transudate; tubercle bacilli, 4.	Yellow; mottled. Fatty infiltration, 3; tubercles necrotic, hyaline; tubercle bacilli, 3.	Measured 3.8×2 cm.; areas of massive necrosis. Tubercles, 3; necrotic; congested; tubercle bacilli, 4.
Immune	19	Distended; pale; tubercles(?) . Pneumonitis, 2; transudate; tubercles, 3; necrotic; tubercle bacilli, 3.	Yellow; mottled. Fatty infiltration, 4; tubercles, 2; tubercle bacilli, 2.	Measured 6.5×3 cm.; diffuse; necrotic; congested. Congested, 4; bacilli, 3. Necrotic; tubercle
Immune	22	Distended; tubercles discrete. Pneumonitis, 4; congested, 2; necrotic; tubercle bacilli, 3.	Yellow; mottled. Fatty infiltration, 1; congested, 3; tubercles, 2, necrotic; tubercle bacilli, 2.	Measured 4×2.5 cm.; massive necrosis. Necrotic, 4; tubercle bacilli, 2.
Immune	28	Distended; tubercles discrete multiple. Pneumonitis, 2; tubercles, 3; necrotic; tubercle bacilli, 4.	Brown; mottled yellow. Fatty infiltration, 1; tubercles, 1, necrotic; congested, 1; tubercle bacilli, 4.	Measured 5×2.5 cm.; dark; multiple necroses. Congested, 2; tubercles, 2, necrotic; tubercle bacilli, 2.
Treated immune	17	Distended; tubercles, o. Pneumonitis, 2; tubercles, 2; transudate; tubercle bacilli, 2.	Light brown; discrete mottling. Fatty infiltration, o; tubercles necrotic and hyaline, 1; congested; tubercle bacilli, 2.	Measured 4×2 cm.; dark mottled yellow. Congested, 2; tubercles necrotic and hyaline, 3; tubercle bacilli, 2.

interval, the pathological findings would have shown striking differences. What stands out strikingly here, however, is the fact that in spite of the overwhelmingly large intravenous injection used as the infecting dose in this experiment, specific immunity displayed a definite retarding effect on the lethal factors involved in tuberculosis, resulting in prolongation of life. These large infecting injections were required to bring out the allergic response with bacillary suspension, and the intravenous injection of 0.01 mg. of virulent bacilli resulted in no allergic deaths. It also appears that the general lethal allergic manifestations do not become significant, at least experimentally, until relatively large amounts of bacilli are involved in the animal economy. Whether general allergy does or does not play a significant part in spontaneous tuberculosis in man is involved with the problem whether the proper antigenic intoxicating materials in man are liberated (or mobilized) *in vivo*, such as the proper mobilization of bacilli or the liberation of tuberculo-proteins. The present evidence would not appear to support the contention that tuberculin (or tuberculo-proteins) as prepared *in vitro* are liberated in active form *in vivo*, although much has been written on auto-inoculation in the past.

SUMMARY AND CONCLUSIONS

The life expectancy of guinea pigs infected subcutaneously or intravenously with highly virulent human tubercle bacilli is prolonged considerably by specific immunization. When large intravenous infecting doses of virulent tubercle bacilli are used, general specific allergic intoxication becomes a significant factor causing an early lethal outcome for the specific immune animals and decidedly lowering the average life span after infection for these animals. By appropriate treatment with suitable filtrates (or pure tuberculins), derived from growing tubercle bacilli on nonprotein mediums, these undesirable allergic lethal intoxications can be prevented. Such treated animals then display only the specific immune protection with resultant retardation of the tuberculosis and prolongation of life. A classical experiment demonstrating the significance of suppressing the specific tuberculo-allergy by appropriate treatment, with resulting prolongation of life of specific immune guinea pigs infected intravenously with virulent tubercle bacilli, is presented. The various phases of the

In analyzing the pathologic information disclosed in the above experiment, several phases are found which merit consideration. The animals that died early as a result of the intravenous injection of the large amount of virulent human tubercle bacilli showed, as would be expected, very little discrete tuberculosis in the lungs, liver and spleen. But they did show predominant evidences of an acute toxicity centered about the general allergic reaction. Yet this was not sufficiently pronounced to present the characteristics of a local reaction resulting from the injection of a sufficient amount of tuberculin to provoke evident tissue changes. The changes noted proved to have more of a secondary character (pulmonary emphysema and congestion of the other organs) in the animals dying early, while later the reaction to the bacilli in the organs obscured this picture so that its recognition was impossible.

It can be assumed, however, from other evidence presented, that the animals that died within the first 10 days following intravenous injection of the virulent bacilli died either from the consequences of the violent specific allergic response or the combination of this with the rapidly developing tuberculosis. This is also borne out by the fact that the controls all died after 11 days and within the short span of 15 to 18 days in most cases, although the part played by specific allergy is difficult to determine. However, when specific allergy in its more active form has subsided as in the immune animals presenting a longer interval, or when it has been suppressed as in the treated immune animals, the predominant reaction seen pathologically is that of the ultimately lethal tuberculosis. The pathological findings in the lungs, livers and spleens at death in the immune or the treated immune guinea pigs do not differ materially from those in the normal infected animals except in so far as can be accounted for by the time that death occurred. Even the bacillary findings cannot be interpreted in this light as significant if we view them with respect to the technical difficulties encountered in such examinations. However, the period of death in this experiment is significant in pointing out the part played by the specific immunity in prolonging life, particularly after the specific allergy has been suppressed in the treated immune animals. As has been shown previously, had the animals all been examined at a certain definite

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DESCRIPTION OF PLATE

PLATE 116

The organic tuberculous involvement at the time of natural death in non-immune and specific immune guinea pigs infected subcutaneously with highly virulent human tubercle bacilli (strain 160).

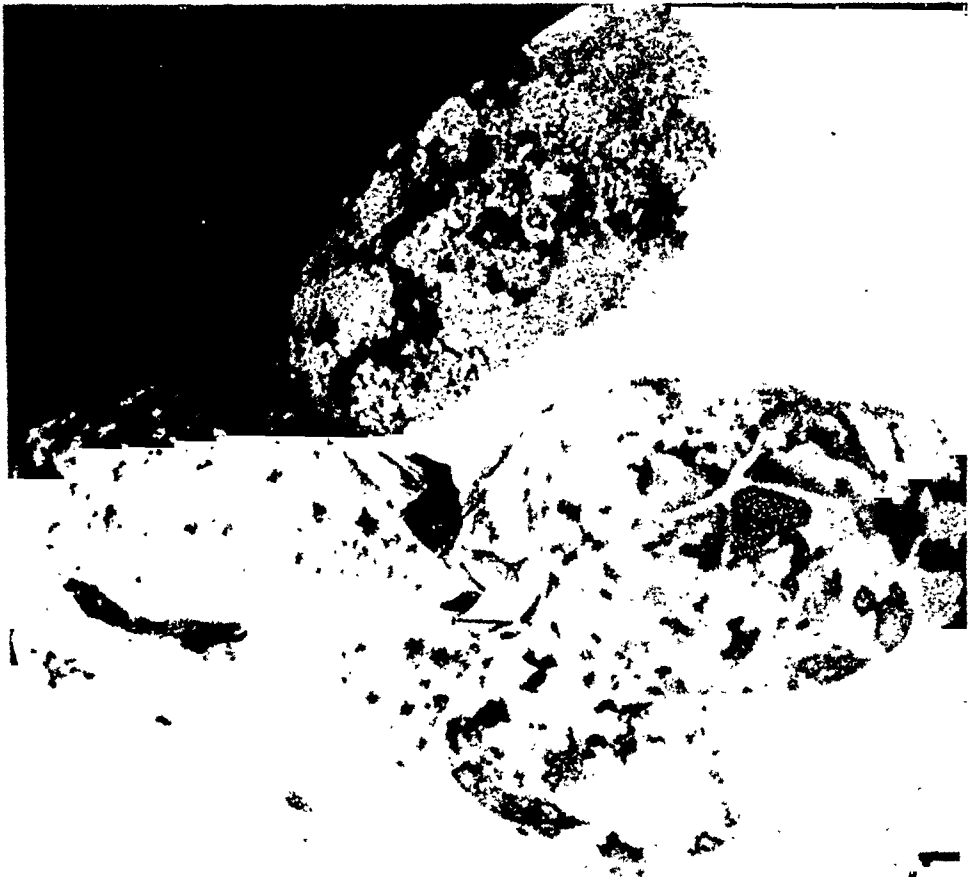
FIG. 1. Control guinea pig, died 103 days after infection with 0.0001 mg. virulent human tubercle bacilli.

FIG. 2. Specific immune guinea pig (prepared by a single subcutaneous injection of avirulent human tubercle bacilli) and infected as was the animal used for Figure 1. This animal died 253 days after infection. Less extensive and more discretely demarcated tuberculosis is shown in the immune animal (Fig. 2) than in the nonimmune control (Fig. 1).

pathology of these conditions in this experiment are also presented. The visual pathological changes at the time of death of these animals disclose only the secondary changes resulting from the specific tuberculo-allergic intoxication on the one hand and the specific tuberculous pathology on the other, unless the time element and other findings are correlated to bring out the significance of the specific immune factors involved. Thus the predominant pathological features noted are the secondary changes produced by the specific tuberculo-allergy, following the second introduction of relatively large amounts of bacillary bodies, and the pathological changes characteristic of tuberculosis as usually recognized.

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Corper

Immunopathologic Features of Tuberculosis

for comparison were selected to test the probability of spread by the several routes mentioned. Since the extent of generalization is popularly believed to be much greater in Negroes than whites, figures were calculated for the races separately in certain of the correlations. The first correlations were to throw light on the amount of spread by passage of infected sputum over the organs concerned. Routinely only one good section of each organ was examined. Additional sections would almost certainly have increased the number of tubercles found. Hence the figures given are minimal.

CORRELATION OF TONSILLAR AND INTESTINAL TUBERCULOSIS

In 126 cases of pulmonary tuberculosis in which the tonsils were examined they were found to be tuberculous in 93 (74 per cent). In 107 cases in which both tonsils and intestines were examined the latter were tuberculous in 84 (79 per cent). Among the 84 cases in which the intestines were tuberculous the tonsils contained tubercles in 68 (81 per cent). In 16 (19 per cent) of the intestine-positive cases no tubercles were found in the tonsils. In the 23 cases in which tuberculosis was not found in the intestines the tonsils were positive for tuberculosis in 57 per cent and negative in 43 per cent of cases. Thus there was a significantly higher proportion of tonsil-positive cases among the intestine-positive than in the intestine-negative cases; yet numerous cases of tuberculosis of the tonsils were found in the absence of tuberculosis of the intestines.

In the 107 cases in which intestines and tonsils were examined the tonsils were tuberculous in 81 (76 per cent) and no tuberculosis was detected in 26 (24 per cent). Among the 81 cases in which the tonsils were positive the intestines were tuberculous in 84 per cent and negative in 16 per cent. In the 26 cases in which tuberculosis was not found in the tonsils the intestines were positive for tuberculosis in 62 per cent and negative in 38 per cent. Thus the percentage of cases with intestinal tuberculosis was significantly higher in the tonsil-positive than in the tonsil-negative group; yet there were numerous cases of tuberculosis of the intestines in the absence of tuberculosis of the tonsils. It must be recalled that the figures are based on gross examination and a single microscopic section from each organ.

THE SPREAD OF TUBERCLE BACILLI BY SPUTUM, BLOOD AND LYMPH IN PULMONARY TUBERCULOSIS *

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Tuberculosis is well generalized in advanced pulmonary tuberculosis. The fact is recognized by most pathologists although minimized in the Ranke concept of tuberculosis, in which the disease is divided into stages, the third of which, typified by phthisis, is defined as isolated organ tuberculosis. The wide generalization is usually unrecognized clinically.

Tubercle bacilli are carried from the lungs in advanced pulmonary tuberculosis by sputum, blood and lymph. A common site of tuberculosis secondary to pulmonary disease, but one that is seldom recognized in life, is the tonsil. One or both members of the pair are tuberculous in the great majority of cases of pulmonary tuberculosis. The lymph nodes tributary to the tonsils are also usually the seat of tubercles in pulmonary tuberculosis. Microscopic study shows minute tubercles in the viscera, often on a scale not greatly different from that in clinical miliary tuberculosis, as well as tuberculosis of the intestines and varying degrees of involvement of the internal and external lymph nodes. Any of the organs named could be infected by either blood or lymph, and two of them, the tonsils and intestines, could be infected by sputum. It has recently been shown that a large proportion of the cases of tuberculosis of the tonsil discovered on the routine examination of this organ after tonsillectomy really represent spread from unrecognized pulmonary tuberculosis.¹

Starting from the latter observation, we have made a study of the several routes of spread by correlating the incidence in selected groups of organs. The material for investigation came from 126 cases of fatal pulmonary tuberculosis, almost exclusively of the common advanced chronic form, occasionally complicated by clinical miliary disease. The 126 cases were selected from a larger group after exclusion of those in which tonsils could not be found on gross examination. The groups of organs set up

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The figures were identical for the opposite correlation of tuberculosis of the intestine and of the mesenteric lymph nodes. Among the 74 positive cases for the mesenteric lymph nodes, tuberculosis was found in the intestine in 66 (89 per cent) and not found in 8 (11 per cent). In the 18 cases in which tuberculosis was not found in the mesenteric lymph nodes, intestinal tuberculosis was present in 44 per cent and absent in 56 per cent. Thus tuberculosis was twice as frequent in the intestine when the mesenteric lymph nodes were tuberculous as when tuberculosis was not found in the nodes.

If it is assumed that intestinal tuberculosis as a complication of advanced pulmonary tuberculosis results as a rule from infection by swallowed sputum, and that tuberculosis of the mesenteric lymph nodes in such cases is secondary to the intestinal disease, resulting from spread by the lymphatics, the figures cited in previous paragraphs indicate equal reason for believing that tonsillar tuberculosis in the presence of pulmonary tuberculosis results from sputum infection and that accompanying tuberculosis of the upper cervical lymph nodes is the result of spread by way of the regional lymphatics from the tonsil to the nodes. Certain European investigators consider tonsillar tuberculosis as usually hematogenous.²

However, in both instances involvement of the tributary organ was noted in the absence of recognized involvement of the source organ. This might have been due to failure to detect the disease in the latter organ, or it might have been from other cause, namely, infection of the respective lymph nodes by another route. The other possible routes are lymphatic from a more distant focus, and hematogenous. The next correlation to be considered throws light on the problem.

CORRELATION OF TUBERCULOSIS OF TONSILS, UPPER CERVICAL LYMPH NODES AND PARATRACHEAL, AXILLARY AND INGUINAL LYMPH NODES

A comparison was made of the incidence of tuberculosis in five lymphoid structures; namely, the tonsils, and the upper cervical, paratracheal, axillary and inguinal lymph nodes. It was assumed that any of these organs might be infected by way of the blood stream and that the various nodes might also be

CORRELATION OF TUBERCULOSIS OF TONSILS AND OF UPPER CERVICAL LYMPH NODES

In 122 cases in which both the tonsils and the upper cervical lymph nodes were examined, tuberculosis was found in the former in 91 cases (75 per cent) and in the latter in 85 (70 per cent). Among the 91 tonsil-positive cases tuberculosis was discovered in the corresponding upper cervical lymph nodes in 76 (83 per cent) and not found in 15 (17 per cent). In the 31 cases in which the tonsils were negative the upper cervical lymph nodes were positive for tuberculosis in 9 (29 per cent) and negative in 22 (71 per cent). Thus tuberculosis was found in the cervical lymph nodes in the majority of cases in which it was present in the tonsils, and in the great majority of cases was not found in the cervical lymph nodes when it was absent in the tonsils.

Among the 85 cases in which the cervical lymph nodes were tuberculous, tuberculosis was discovered in the corresponding tonsils in 76 (89 per cent) and not found in 9 (11 per cent). Among the 37 cases in which tuberculosis was not found in the upper cervical lymph nodes, tubercles were discovered in the tonsils in 15 (41 per cent) and not found in 22 (59 per cent). Thus tuberculosis was present in the tonsils in only a minority of cases in which it was absent in the upper cervical lymph nodes.

CORRELATION OF TUBERCULOSIS OF INTESTINES AND OF MESENTERIC LYMPH NODES

In 92 cases in which both intestine and mesenteric lymph nodes were examined tuberculosis was found in the former in 74 (80 per cent) and in the latter, also, in 74 (80 per cent). Among the 74 intestine-positive cases tuberculosis was found in the mesenteric lymph nodes in 66 (89 per cent) and not found in 8 (11 per cent). Among the latter were several cases with such extensive amyloidosis that tuberculosis could not have been detected in the lymph nodes. In the 18 cases in which tuberculosis was not found in the intestine the mesenteric lymph nodes contained tubercles in 8 (44 per cent) and no tuberculosis was found in 10 (56 per cent). Thus tuberculosis was twice as frequent in the mesenteric lymph nodes in cases of intestinal tuberculosis as in cases free from intestinal tuberculosis.

(18 per cent). If the latter figure is taken as a rough index of the hematogenous involvement of the various lymph node groups, the difference between this figure and the amount found may be taken as a rough measure of the amount of infection by lymphatic spread from neighboring tuberculous structures. It will be shown later that the incidence of hematogenous infection of the spleen and liver was much higher than the figure for the inguinal nodes, but the latter seems justifiably used for the comparison of infection in lymph nodes.

It is interesting to note that tuberculosis of the axillary nodes was detected in one-third of the cases examined. Frequently, the small tubercles found were central, suggesting hematogenous origin. About equally commonly they appeared peripheral in origin, as if the result of lymph-borne infection. In approximately two-thirds of the cases the upper cervical lymph nodes were tuberculous. If the rough measure of hematogenous infection, namely, 18 per cent, is subtracted from the incidence in the axillary nodes and the upper cervical nodes, without allowing for the number of cases that might have been infected by both routes, the latter are seen to have been involved about three times as commonly as the former. This would seem to rule out the likelihood that much if any of tuberculosis discovered in the upper cervical nodes was the result of lymphatic extension from the lungs by way of pleural adhesions.

The incidence of tuberculosis of the high paratracheal nodes was approximately the same as that found in the upper cervical nodes. Whether retrograde drainage from the former to the latter group took place in an appreciable number of cases cannot be determined. In view of the apparent relative infrequency if not actual absence of spread from the summit of the lung, however, as indicated in the preceding paragraph, spread by this retrograde route from the more remote paratracheal to the upper cervical nodes seems unlikely.

Since lymphatic and hematogenous spread of tuberculosis is believed to be more conspicuous in the Negro than in the white race, advantage was taken of the material available to test this conception. Of the 85 patients in whom the series of organs noted was examined, 26 were white and 59 were Negro. These figures are too small for accurate statistical comparison, but it is at least

infected by extension through the lymphatics from neighboring infected organs. Specifically the upper cervical nodes might be infected from the tonsils and the paratracheal and axillary nodes from the lungs. The paratracheal nodes selected were those at the level of the clavicle, still in the line of drainage from the lungs to the thoracic duct. Infection other than hematogenous would be from lymph that had already passed through several lymph node filters, which in turn had been infected. Axillary lymph node infection might be hematogenous or the result of spread of tuberculosis through the lymphatics of pleural adhesions. Bilateral pleural adhesions were practically universal in the series of cases studied. But, if tuberculosis could spread by this route to the axillary nodes, it could spread to the neck by the same route and infect the lower cervical nodes. With infection of the lower nodes, disturbance in flow with retrograde diversion of lymph might occur, leading to infection of the upper cervical nodes. Thompson,² quoting the pertinent literature, has described this route of infection in more detail. Thus it is conceivable that tuberculosis of the latter group might result by lymphatic spread from the lungs. With such considerations in mind it seemed desirable to make a unilateral comparison of the incidence of tuberculosis in the axillary and upper cervical nodes.

Of all the lymph node groups selected the one most nearly representing hematogenous infection alone was the inguinal group. The inguinal nodes could be, and several times in the series were, infected as part of a generalized lymphatic dissemination, but for the most part were uninfected or were the seat of isolated tuberculosis of blood-borne origin. It is recognized that occasionally hematogenous infection might be indirect, by lymphatic spread from a part below the groin previously infected hematogenously, and that the lymphatics of a very small portion of the lower bowel drain to the superficial inguinal lymph nodes.

All five groups of organs, *i.e.*, tonsils and upper cervical, paratracheal, axillary and inguinal lymph nodes, were examined in 85 cases. In 63 cases (74 per cent) the tonsils were tuberculous. In 54 (64 per cent) the upper cervical nodes on the same side as the infected tonsils were tuberculous. In 53 (63 per cent) the paratracheal nodes were tuberculous. The axillary nodes were infected in 27 (32 per cent) and the inguinal in 15

cal lymph nodes on the same side of the body were tuberculous in 71 cases (70 per cent). Tuberculosis was found in 70 cases (69 per cent) in the spleen, in 61 cases (60 per cent) in the liver, in 23 cases (23 per cent) in one or both kidneys and in 16 cases (16 per cent) in one or both adrenal glands.

The incidence of visceral involvement corresponds with that commonly reported. The spleen and liver were tuberculous in 60 to 70 per cent of cases and the kidney and adrenal with far less frequency. The spleen appears to be the most sensitive indicator of the spread of tuberculosis by way of the blood stream. It must be recalled that the figures given in this study are minimal figures, in that, as a rule, only one section was examined. Presumably the bacilli that reach the spleen enter almost exclusively through the splenic artery, but they appear to find a medium unusually suitable for growth. The liver, with a relatively smaller arterial supply, draws blood from the portal vein and may be tuberculous as a result of dissemination from intestinal lesions, which were present, as noted, in about three-fourths of the cases in this investigation. The kidneys, with a greater arterial blood supply than the spleen, were much less frequently tuberculous than the latter, although more frequently tuberculous (23 per cent of cases) than is commonly assumed. The adrenals, with the richest arterial blood supply of all, were discovered to be tuberculous in but 16 per cent of cases.

In large measure the likelihood of an organ becoming tuberculous is dependent upon a slow speed of circulation and the presence of phagocytic cells which may remove bacilli from the blood stream, but fail to destroy them. The spleen and liver are vulnerable in these respects, even though these organs probably destroy a large percentage of the tubercle bacilli that reach them (Lurie^{3,4}). The kidney has relatively few macrophages, but anatomically is a filter with a tortuous circulation, and for this structural reason may be expected to trap bacilli travelling in large cells. The adrenal glands are not richly supplied with phagocytic cells to remove bacilli, nor traplike in structure, facts probably accounting for the low incidence of tuberculosis in this organ as compared with the other viscera in the series here under consideration.

Again, because of the common conception of excessive fre-

of interest to note that no great difference was found in the incidence of involvement of the different organs in the two races. The inguinal nodes, believed to represent hematogenous infection chiefly, were tuberculous in 5 out of 26 cases (19 per cent) in the whites and 10 out of 59 cases (17 per cent) in the Negroes. The axillary nodes, apparently representing in their tuberculosis lymphatic and hematogenous extension about equally, were tuberculous in 10 out of 26 cases (38 per cent) in the whites and in 17 out of 59 cases (29 per cent) in the Negroes. The upper cervical nodes, probably representing chiefly spread from the tonsil and to a lesser degree hematogenous dissemination, with lymphatic spread from the lungs an unknown if existent value, were tuberculous in 18 out of 26 cases (69 per cent) in the whites and 36 out of 59 cases (61 per cent) in the Negroes.

Thus there was no evidence of a greater degree of hematogenous and lymphatic dissemination of tuberculosis in the lymph node series in Negroes than in whites.

An additional analysis was made of the part played by sex in the Negroes. As is well known, the mortality from tuberculosis is exceptionally high in young Negro women. Of the 59 Negroes, 30 were male and 29 female. The average age for males was 34 and for females 28 years. The inguinal nodes were tuberculous in 6 out of 30 cases (20 per cent) in males and 4 out of 29 cases (14 per cent) in females. The axillary nodes were tuberculous in 9 out of 30 cases (30 per cent) in males and 8 out of 29 cases (28 per cent) in females. The upper cervical nodes were tuberculous in 17 out of 30 cases (57 per cent) in males and 19 out of 29 cases (65 per cent) in females. Thus no significant difference between the sexes was noted.

CORRELATION OF TUBERCULOSIS OF THE TONSILS AND UPPER CERVICAL LYMPH NODES, AND SPLEEN, LIVER, KIDNEYS AND ADRENALS

Further analysis was made of the rôle of hematogenous infection by examining the tonsils and upper cervical lymph nodes, the organs primarily under consideration in this study, in comparison with four visceral organs, namely, the spleen, liver, kidneys and adrenals. In 102 cases all six sets of organs were studied. The tonsils were tuberculous in 76 cases (75 per cent). Upper cervi-

man, 24 years old, had typical gross miliary tuberculosis as a terminal process superimposed on chronic pulmonary tuberculosis. The pancreatic tubercles were found in a girl of 12 years and a man of 48 years. In each case tuberculosis was widely generalized, but not typically miliary in gross character. The tubercles noted in several instances were in the depths of the two organs. Inward extension from tuberculous pericarditis was not counted as tuberculosis of the myocardium, nor was tuberculosis of peripancreatic nodes immediately adjacent to the pancreas counted as tuberculosis of the latter.

Generalized amyloidosis and fatty infiltration of the liver were frequently present in this series. There were 8 cases of amyloidosis, or 8.3 per cent of the 96 cases, 4 of them in white patients and 4 in Negroes. The figures are too small for valid statistical comparison, but the 4 cases in whites constituted 15 per cent of the white patients and the 4 cases in Negroes only 6 per cent of the Negro cases. In all cases in which there was generalized amyloidosis the intestine was tuberculous.

Advanced fatty infiltration of the liver was noted in 25 of the 96 cases (26 per cent). The figures for tuberculosis in whites and Negroes were not significantly different; namely, 31 per cent for whites and 24 per cent for Negroes.

SUMMARY

A series of 126 cases of pulmonary tuberculosis was studied to determine the relative parts played by sputum, blood and lymph in the dissemination of tuberculosis throughout the body. The tonsils and intestine were chosen as measures of infection by sputum, and the upper cervical, paratracheal, axillary, mesenteric and inguinal lymph nodes as measures of lymphatic spread, with recognition of the fact that all of the organs named may be infected hematogenously. Hematogenous spread was studied in the spleen, liver, kidneys, adrenals, myocardium and pancreas.

Figures are given showing the frequency of infection in the several organs named. A high positive correlation existed in tuberculosis of the tonsils, upper cervical lymph nodes, intestine and mesenteric lymph nodes, presumably indicating sputum infection of the organs of the alimentary tract and secondary involvement of the draining lymph nodes by the lymph stream. Of the

quency of hematogenous invasion in Negroes, a comparison was made of the incidence of tuberculosis in the several organs in the two races. At this point it should be noted that no sharp separation of miliary tuberculosis in the series studied could be made microscopically. A few of the cases were diagnosed grossly as miliary tuberculosis terminal to pulmonary tuberculosis, but many of the cases not designated as miliary grossly, exhibited extensive generalization of tubercles microscopically.

Of the 102 cases in the series, 29 were of white and 73 of Negro patients. There was approximately equal division of the sexes in the two groups. The tonsils were tuberculous in 23 out of 29 cases (79 per cent) in the whites and 52 out of 73 cases (71 per cent) in the Negroes. The upper cervical lymph nodes were tuberculous in 65 per cent of cases in whites and 70 per cent of cases in Negroes. The spleen was tuberculous in 65 per cent of cases in whites and 67 per cent in Negroes. For the liver the figures respectively for whites and Negroes were 55 and 62 per cent. For the kidneys they were 21 and 23 per cent and for the adrenals 17 and 16 per cent. Thus, by this comparison also there was no evidence that Negroes are more susceptible to hematogenous dissemination of tubercle bacilli than whites.

CORRELATION OF TUBERCULOSIS OF SPLEEN, LIVER, KIDNEYS, ADRENALS, MYOCARDIUM AND PANCREAS

A final study of hematogenous invasion was made by selecting for comparison those cases of the total 126 necropsied in which examination was made of the myocardium and pancreas as well as the spleen, liver, kidneys and adrenals. There were 96 such cases. The frequency of tuberculosis decreased in the following order: spleen, 64 cases (67 per cent); liver, 58 cases (60 per cent); kidneys, 24 cases (25 per cent); adrenals, 16 cases (17 per cent); myocardium, 2 cases (2 per cent); and pancreas, 2 cases (2 per cent).

It is perhaps noteworthy that the 4 cases in which tuberculosis was found in the myocardium or pancreas were all in Negroes. Tubercles of apparent hematogenous origin were found in the myocardium in Negro males of 17 and 24 years. The boy of 17 years had extensive generalization of tuberculosis with an old encapsulated primary lesion in a mesenteric lymph node. The

several series of lymph nodes examined, the superficial inguinal nodes were infected the least frequently. Their incidence of tuberculosis was considered a rough measure of the extent of hematogenous spread to the lymph nodes, which could be subtracted from the total incidence of infection of the other lymph nodes to furnish a rough minimal index of the involvement of the latter by way of the lymph stream.

The tonsils and intestine were tuberculous in about three-fourths of the cases, and the upper cervical and mesenteric lymph nodes in almost three-fourths. Tubercles were found in the axillary nodes in one-third and in the inguinal nodes in more than one-sixth of the cases of the series.

A striking difference in the incidence of hematogenous infection was apparent on examination of the viscera. Single section examination showed tubercles in approximately two-thirds of the cases in the liver and spleen, in one-fourth in the kidney, 16 per cent in the adrenal and 2 per cent each in the myocardium and pancreas.

Although tuberculosis is commonly believed to exhibit a greater tendency to hematogenous and lymphatic spread in Negroes than in whites, no significant difference between the races was found in these respects in the series here reported, nor was any difference found between the sexes.

The incidence of generalized amyloidosis was 8 per cent and of advanced fatty infiltration of the liver, 26 per cent. No racial difference was apparent.

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Microscopically, three definite zones can be identified in the wall (Fig. 2). The inner lining of the cavity is a soft gray, pyogenic membrane. Beneath this membrane is a zone of granulation tissue with numerous dilated capillaries, many lymphocytes and large mononuclear cells which in the chronic forms are replaced by fibrous tissue. The latter is never as thick nor as dense as the mature hyaline tissue about cavities of tuberculo-silicotic origin (Fig. 4). Occasional silicotic nodules, incorporated in the wall, may be partially eroded by the ulcerative process or they may exhibit a caseous center surrounded by a layer of inflammatory cells. The concentric laminae of thick hyaline fibers distinguish these nodules from the more irregular tubercles that usually occur in the immediate vicinity. For details of differentiation reference is made to the recent publication by Gardner.¹

Tubercle bacilli can be easily demonstrated in the contents or wall of these excavations. Their tinctorial and cultural characteristics and their virulence for guinea pigs are similar to those of bacilli of human type recovered from lesions of uncomplicated pulmonary tuberculosis.

The origin and development of such cavities are essentially the same as those of uncomplicated pulmonary tuberculosis. Although the process may be accelerated by the presence of silica in the lung, the pathology and clinical manifestations of the infectious element remain unaltered. Either the amount of silicosis or the state of activity seems to have been insufficient to modify the course of the associated infection.

TUBERCULO-SILICOTIC CAVITIES

Excavations of this type (Fig. 3) are invariably found within areas of massive, hard, black to gray conglomerate fibrosis. Most of them are small and unilocular. The thick, dense fibrous tissue surrounding them apparently resists necrotizing action and prevents the extension productive of multilocular forms. The content of these cavities is purulent, but inky-black. Occasionally small gray-black masses of silicotic fibrous tissue are found free in the pus. Some of them represent sequestered nodules eroded from the wall in the process of tissue degeneration. The trabeculation, so common in the uncomplicated tuberculous cavity, is seldom seen, probably because the blood vessels are no more

CAVITIES IN THE SILICOTIC LUNG*

A PATHOLOGICAL STUDY WITH CLINICAL CORRELATION

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The presence of a cavity in any lung has a serious clinical import, not only because it represents destruction of pulmonary tissue, but because it serves as a source of numerous complications. In the silicotic lung more cavities are observed at the autopsy table than are recognized during life by X-ray or physical examination. Pathological investigation has shown that all of these cavities are not alike and it is possible to differentiate three distinct types:

1. Cavities associated with a *typical tuberculosis* that is little modified by coexistent silicosis.
2. Cavities occurring in areas of *tuberculo-silicosis*, an extremely chronic condition resulting from the combined local effect of tubercle bacilli and silica dust. In them, the results of infection are obvious and tubercle bacilli are usually demonstrable.
3. Cavities of the so-called *anemic type* which develop within areas of massive fibrosis, but show no evidence of causative organisms or cellular reaction.

TYPICAL TUBERCULOUS CAVITIES

Cavities closely resembling those found in uncomplicated tuberculosis (Fig. 1) usually occur in cases with minimal silicotic nodulation. Such cavities may be large or small, unilocular and spheroidal or multilocular with numerous extensions into the surrounding lung. They usually contain a thick purulent liquid which varies in amount depending upon the patency of the draining bronchus. A wall of inflammatory tissue demarcates them from the air-containing lung. The structure of their wall varies with the chronicity of the lesion. In the rapidly progressing types it is soft and pliable, whereas in those that develop more slowly and have existed for a long time the wall is usually firm and rigid.

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pig inoculation or culture, however, usually demonstrates their presence.

Other features of the massive wall about the tuberculo-silicotic cavity are also significant. The hyaline, nodular components of the lesion can usually be identified in regions least involved by the tuberculous process. In some of the mature reactions, however, even this feature may be obscured since the contraction of the heavily pigmented matrix draws the nodules close together and distorts their boundaries, ordinarily clearly defined.

The pigmentation is due to the particles of nonsiliceous minerals that were inhaled with the free silica in the working atmosphere. In the case of coal this consists of black carbonaceous material; the associated oxides of iron, magnetite and hematite, respectively cause a black or red pigmentation. More attention should be given to this nonsiliceous component of the inhaled dust because of its effect upon the inflammatory response to the particles of silica. Experimental studies have shown that coal and these oxides of iron retard and modify the usual reaction to silica in the pure form.² The mechanism of this inhibitory action has not been completely established.

In an area of massive fibrosis practically all the normal pulmonary structures are involved. The blood vessels reveal an advanced obliterative arteritis and phlebitis. The bronchi and bronchioles are markedly compressed and distorted by the surrounding fibrosis and by the inflammatory changes within their walls. The epithelial lining is frequently desquamated, but where present it shows no evident hyperplasia.³ The submucosa is thickened by granulation tissue and occasionally contains small proliferative tubercles.

The intrapulmonary and extrapulmonary complications of ordinary tuberculous cavities rarely accompany excavations of the tuberculo-silicotic type. Hemorrhage may occur as a late manifestation, but usually the avascularity of the wall prevents the repeated hemoptyses so common in uncomplicated ulcerative phthisis. The same factor undoubtedly retards hematogenous dissemination of bacilli, for miliary tuberculosis is uncommon. Spread by aspiration throughout the lungs, and also tracheobronchial, laryngeal and intestinal complications, are all rare.

The evolution of the tuberculo-silicotic cavity in the living sub-

resistant to the very slow process of necrotization than the very dense fibrous tissue that surrounds them.

The most important feature of these cavities is the zone of dense black fibrous tissue which surrounds them. The usual internal necrotic membrane (Fig. 4) is extremely thin and may line only a portion of the cavity. It often blends imperceptibly with the overlying pigmented scar tissue and does not exhibit the pronounced pyosis so common in simple tuberculous excavations. It is probable that in the early stages of such cavities there may be considerable infiltration with leukocytes, but as they become older the avascularity which is concomitant with massive fibrosis retards migration of such cells from the blood stream. As a consequence the lining membrane simulates that observed in simple necrosis rather than in tuberculosis.

A conspicuous feature is the absence of an intermediate zone of hyperemic granulation tissue, which is so characteristic of simple ulcerative phthisis. Although the microscope may reveal granulations in an occasional minute portion of the wall, such areas are few and poorly vascularized.

The zone of conglomerate fibrosis surrounding the cavity may be only 2 to 3 cm. in thickness, but more often it involves a large part of a lobe and may even cross the fissure into the neighboring lobe. With extension to the periphery of the lung, the pleura is thickened and firmly attached to the adjacent chest wall by focal adhesions or an obliterating fibrous pleurisy.

The tuberculous components of the conglomerate lesion, apart from the cavity, may be difficult to identify since they are modified by the tissue response to the particles of silica. The foci of caseation scattered throughout the mass are generally smaller and firmer than in ordinary tuberculosis. Microscopically, there are usually areas in which hyaline bands of fibrous tissue are poorly stained and appear swollen and hazy in outline. In and about such areas there is often an infiltration of inflammatory cells, but these are rarely concentrated in foci to form tubercles.

The histopathological characteristics of the complicating tuberculosis are so atypical that in some instances bacteriological methods must be employed for diagnosis. Acid-fast bacilli are so sparsely scattered throughout the massive wall of the cavity that often they cannot be found on direct examination. Guinea

but as compared with the exudate in tuberculous cavities it is more fluid and not purulent. The well defined inner zone of purulent material that lines tuberculous cavities is absent. In its place is an indefinite, ragged zone of seminecrotic tissue of the same color as the surrounding fibrosis. There are no trabeculae. The thick limiting wall of black fibrous tissue shows nothing suggestive of tuberculosis.

Microscopic sections (Fig. 6) reveal ends of fibrous strands terminating abruptly at the lumen of the cavity. Often small degenerated fragments hang into the cavity and because of their indefinite structure and poor staining quality they appear as ghost strands. The dust cells usually found between the fibers are disintegrated and their particles lie scattered throughout the area. More distant from the cavity, the massive wall is uniformly a heavily pigmented fibrous tissue of the type commonly seen in anthracosilicosis or in reactions to other mixtures of free silica with various minerals.

The blood vessels within the mass show arteritis and phlebitis of a high grade. The earliest changes recognizable in the arteries are a thickening of the intima and a narrowing of the lumen by a new formation of connective tissue. With further advance of the disease the whole structure is transformed into a dense connective tissue containing scattered dust particles. At this stage the vessel can no longer be differentiated from the surrounding fibrosis except perhaps by elastic tissue stains. The veins are also involved, but in the more chronic lesions they cannot be identified.

The remarkable feature about these excavations, setting them apart from the tuberculous cavities, is the absence of histopathologic and bacteriologic evidences of infection. Capillary dilatation, edema and leukocytic infiltration with the formation of an inner pyogenic membrane are absent. Tubercle bacilli cannot be demonstrated either by staining or animal inoculation.

These cavities result from a combination of factors, of which the most important appears to be vascular. Both roentgenographic studies of the lungs after injection of radio-opaque material into the vascular tree and histological studies demonstrate that the blood vessels in the massive fibrotic areas are practically obliterated. The resulting anemia in the depth of the lesion is fol-

ject is hard to follow. The large masses of fibrous tissue, in which such cavities develop, form very slowly. Such lesions are so dense that they are not readily penetrated by the X-ray, with the result that even the specific evidences of tuberculosis are hard to recognize. Because of the delayed appearance of a positive sputum and of symptoms referable to tuberculosis, no warning is given of changes that may be taking place within areas of massive fibrosis. Progression of a primary infection to the stage of cavity formation is conceivable. The Saranac Laboratory museum has one case in which this mechanism seems probable. Ordinarily, however, the pulmonary portion of the primary complex of childhood no longer contains living organisms by the time dust exposures begin. Primary infections acquired later, after silicosis has developed, tend to run an acute course resulting in the so-called "perinodular tuberculosis." Such cases terminate in tuberculous pneumonia which may be complicated by rapid formation of cavities, but these lesions are outside the scope of this paper. The ordinary chronic cavity of tuberculo-silicosis is probably the result of a reinfection whose source may be either exogenous or endogenous. If of the latter origin it may result from the reactivation of a latent encapsulated focus of infection.

The resistance of the host and the number and virulence of the infecting organisms play a part, but one of the chief factors is an active silicotic lesion. In experimental lesions which are in a stage of necrosis, tubercle bacilli multiply with unusual rapidity.⁴ In animals it has been shown also that latent tuberculous lesions, which in normal hosts tend to heal and disappear, will become reactivated and spread under the influence of subsequently inhaled silica.⁵ These results follow without reference to the native or artificially induced resistance in the host.⁶

ANEMIC CAVITIES

As in tuberculo-silicosis, cavities of anemic type (Fig. 5) also are found within pigmented areas of conglomerate fibrosis. They are differentiated from those in tuberculo-silicosis by the following characteristics. The cavities are usually small, elongated and slitlike. In a few cases, however, they have been of considerable size, occupying a large portion of a lobe. Their content is inklike,

CLINICAL CORRELATION

The *typical tuberculous cavities* are in no way unusual in the subject with early discrete nodulation. The clinical picture is the same as that in simple chronic phthisis and the lesions are associated with the common pulmonary and extrapulmonary complications. The clinical problem in these cases is primarily one of diagnosing the concomitant silicosis. The X-ray is the most reliable means of differentiating silicotic nodules from tubercles of bronchogenic or hematogenous origin. In cases with advanced infection differentiation may be extremely difficult or impossible.

In the tuberculo-silicotic subject the clinical problem is more difficult. The modifying effect of the silicosis upon the tuberculous element in this disease alters the picture so that the symptoms exhibited are characteristic of neither of the conditions in uncomplicated form. For this reason the condition of *tuberculo-silicosis* must be regarded as a separate disease entity.

Frequently it requires an over-exposed film to demonstrate the presence of a cavity in the center of an area of massive fibrosis. The characteristic signs and symptoms usually accompanying excavations in chronic phthisis are often absent. The peculiarities of the pathology of tuberculo-silicosis with cavity formation will explain this apparent paradox. The pronounced avascularity not only of the fibrous tissue immediately about the excavation, but also throughout the surrounding thick zone of hyalinized scar, retards the absorption of toxic products elaborated at the site of the cavity. As a result, these patients seldom experience the marked loss of weight, the weakness and the elevated temperature, or other toxic manifestations of uncomplicated pulmonary tuberculosis. The absence of the hyperemic zone in the cavity wall also explains the rarity of other complications, such as hemorrhage and hematogenous spread of disease, that commonly accompany simple tuberculous cavities. The compression and occlusion of the air passages by a mass of rigid scar tissue retard the drainage from the cavity so that the sputum often remains negative for tubercle bacilli until late in the course of the disease. In a few cases repeated examinations have failed to demonstrate bacilli throughout life although after death they were found in the wall of the cavity.

lowed by areas of local necrosis which because of autolysis ultimately liquefy and excavate.

Other possible factors which must be considered in the etiology of such necrosis and excavation are the toxic action of silica and the modifying influence of the nonsiliceous dust that usually contaminates the lesion. Both clinical and experimental evidence indicates that silica in high concentration is toxic and kills tissue. The nonsiliceous dusts may be concentrated in such areas simply because the fibrosis has occurred, or it is possible that the heavily pigmented tissue is actually more sensitive to a deficient blood supply. As a matter of observation, necrosis, liquefaction and resultant excavation begin in pigmented scar tissue and ultimately involve all of the structures in the immediate vicinity.

LOCATION OF CAVITIES

Table I compares the location of the cavities in 339 cases of uncomplicated tuberculosis with those in 94 cases of silicosis. The latter are subdivided into typical tuberculous cavities in silicotic lungs (34 cases) and tuberculo-silicosis (60 cases).

It will be noted that in all three types of disease cavities may occur in any part of the lung, but in all they are most frequent in

TABLE I
Comparison of Location of Cavities in 339 Cases According to Type

Lobe of lung	Simple tuberculous cavities	Typical tuberculous cavities in silicotic lungs	Tuberculo-silicotic cavities
	(per cent)	(per cent)	(per cent)
Upper	76	67	62
Middle	7	9	8
Lower	17	24	30

the upper lobes. In the lower lobes the incidence is nearly twice as great in the tuberculo-silicotic cases as in those with uncomplicated tuberculosis. The frequency of middle lobe involvement is approximately the same in all three categories. A point of difference, not indicated in the table, is a predilection toward localization in the midportion and lower portion of each lobe of the silicotic lung. In nonsilicotic subjects more cavities occur in the posterior portions of the lobes.

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DESCRIPTION OF PLATE

PLATE 117

- FIG. 1. Tuberculous cavity in a silicotic lung. The wall is thin and contains scattered, deeply pigmented, silicotic nodules. $\times 0.85$.
- FIG. 2. The wall of the tuberculous cavity showing a well developed pyogenic membrane, capillary lumina in the granulation tissue zone and a peripheral zone of non-silicotic fibrous tissue. $\times 45$.
- FIG. 3. Tuberculo-silicotic cavity occupying the middle lobe. The interlobar fissures are obliterated. The component nodules of the massive conglomerate lesion are easily identified. $\times 0.4$.
- FIG. 4. The wall about the tuberculo-silicotic cavity. The pyogenic membrane is thin and blends imperceptibly with the hyalinized fibrous tissue responding to silica. An intermediate zone of hyperemic granulation tissue is absent. $\times 45$.
- FIG. 5. Anemic cavity within a massive conglomerate lesion of silicotic fibrous tissue. The scar obliterates the fissure and involves a portion of the lower lobe. $\times 0.4$.
- FIG. 6. The wall immediately about the anemic cavity. It is lined by an indefinite ragged zone of seminecrotic tissue. A pyogenic membrane is absent. $\times 45$.

Because of these peculiarities, cases of tuberculo-silicosis seldom develop early spread of the disease either by contiguous extension or through the bronchial tree. Secondary complications such as tuberculous tracheobronchitis, laryngitis and enteritis are much less frequent than in ordinary tuberculosis.

The *anemic cavities* offer very few clinical features for correlation. Usually the excavations are so small that they cannot be differentiated from the surrounding mass of conglomerate fibrosis either by roentgenographic methods or other means at the disposal of the examining physician. When demonstrated, they are most often interpreted as tuberculous, since their location is not unlike that of the tuberculo-silicotic cavities.

SUMMARY AND CONCLUSIONS

This paper summarizes the pathologic features and associated clinical picture of three types of cavities commonly encountered in the silicotic lung.

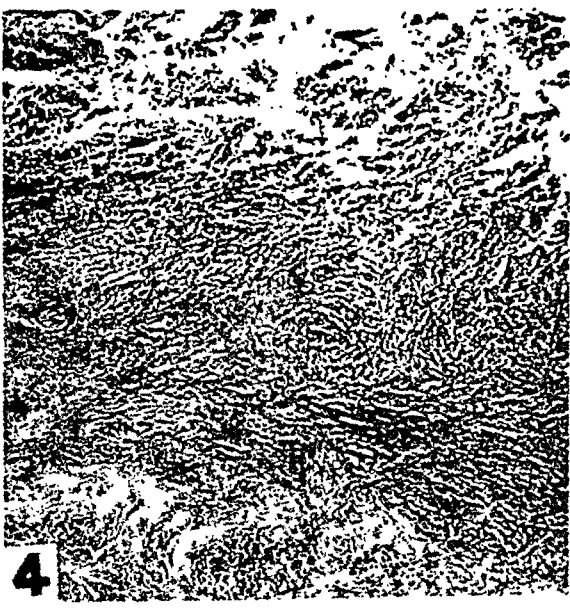
Typical tuberculous cavity formation develops most often in persons with minimal silicosis. It is characterized by the ordinary pathological and clinical features of excavation in uncomplicated chronic phthisis.

In the massive fibrotic lesions of tuberculo-silicosis, produced by the combined local action of silica and tubercle bacilli, cavities of modified form usually develop. They differ in morphology from the simple tuberculous cavities and do not give rise to the usual symptoms until the disease has existed for a very long period.

Anemic cavities also may occur within massive lesions of conglomerate fibrosis without evidence of active infection. They are probably due to local deficiency in blood supply, although the toxic action of high concentrations of silica may play a part. Of themselves, they do not cause recognizable clinical symptoms.

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formed, but seldom persisted for more than a fortnight. Never did a local abscess occur. On January 1, 1940, the director mailed a questionnaire to the foster parents of 1,434 injected children. From these 1,110 replies were received. The average age of the children at that time was about 4 years. Of the 91 who had had pertussis, 68 were in families having no other children.

For controls, Dr. James P. Campbell, of the Geneva Community Hospital, compiled data on the attack rate of pertussis in children born in the obstetrical department between 1933 and 1939. In this hospital, which is located 45 miles from Chicago, somewhat fewer than 200 infants are born annually. On January 1, 1940, the superintendent mailed a questionnaire to the parents of 1,000 living children born there during the previous 7 years. From these 464 replies were received. The average age of the children at the time of the investigation was somewhat less than 4 years. Forty-two were in families having no other children. Vaccine had not been given to 256; 208 had had authorized *H. pertussis* vaccine after the age of 7 months. No Geneva child injected since 1935 has developed whooping cough. In Table I the attack rates of the control group (no vaccine) and the "Cradle" group are compared with the attack rates of three groups injected after the sixth month. The attack rate of the injected Geneva group coincides with the attack rate of the Evanston Health Department clinic group and is lower than that of Evanston privately injected older infants.

Before the end of 1934 and in each year since then, children injected with the vaccine made in 1934 have developed the disease. Many of these cases can be attributed to the impotency

TABLE I
Age Factor in Active Immunization Against Pertussis

Group	Period	Age when injected	Number of infants	Had whooping cough	Attack rate
		<i>months</i>			<i>(per cent)</i>
Geneva (controls—no vaccine)	1933-39		256	38	14.84
Cradle	1933-39	2 to 3	1110	91	8.19
Geneva private	1933-39	over 7	208	3	1.44
Evanston clinic	1934-39	over 8	1586	20	1.26
Evanston private	1933-39	6 to 8	1206	30	2.48

THE AGE FACTOR IN ACTIVE IMMUNIZATION AGAINST WHOOPING COUGH*

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Because whooping cough is fatal only during the first 2 years of life, and since it causes more infant deaths than do the other preventable contagious diseases—diphtheria, scarlet fever and smallpox—active immunization, if it is to be effective, should be attempted as early as immunity can be conferred. The vaccine should not elicit severe local or systemic reactions; nor should it sensitize the individual. Authorized† *Haemophilus pertussis* vaccine referred to in this report was made with human blood; it was not “washed” because it contained no animal protein.

Two opportunities presented themselves for obtaining controlled data on the use of authorized *H. pertussis* vaccine as an immunizing agent in the first months of life. One was at the Evanston “Cradle,” a placement shelter for newborn infants; the other was at the four infant welfare stations of the Chicago Health Department where the highest incidence of deaths from pertussis had occurred.

In 1933, the director of the “Cradle” requested the injection of each thriving infant more than 1 month of age and weighing more than 7 lbs. Although it was suggested at the time that if alternate infants were injected, the uninjected would serve as controls, controls from another source were established instead. Authorized *H. pertussis* vaccine was used. At first, each infant was injected with 1 cc. weekly in alternate arms for 6 successive weeks, so that a total of 60,000 million bacilli was injected. Not until 1934 was the customary total dosage of 80,000 million bacilli given to these young infants. About 200 “Cradle” infants less than 3 months of age were injected each year for 7 years. These very young infants withstood the injections remarkably well. The rectal temperature seldom rose above 38.5° C.; the local reaction was usually transient. A subcutaneous nodule

* Received for publication March 26, 1941.

† Prepared from recently isolated strains of Phase I pertussis bacilli, according to detailed specifications furnished by Northwestern University Medical School.

cine. Of these tests 65 were negative; 10 were +; 13 were ++; and 1 was +++. Similar tests and retests on older infants, performed from 1 to 12 weeks after the final vaccine injection, were usually positive; in over 70 per cent the test was +++ or +++++.

The intracutaneous pertussis test was performed on 35 "Cradle" infants from 1 to several weeks after the final vaccine injection. One part of the clear supernatant fluid (after prolonged centrifugation of 15,000 million bacilli per cc. authorized vaccine) was diluted with fifteen parts of 0.5 per cent phenol in 0.85 per cent sterile saline. When 0.1 cc. was injected intracutaneously, the test was negative when observed 24 hours later. When performed in older infants, from 1 to 12 weeks after the final vaccine injection, an erythematous area at least 10 mm. in diameter was usually observed 24 hours later.

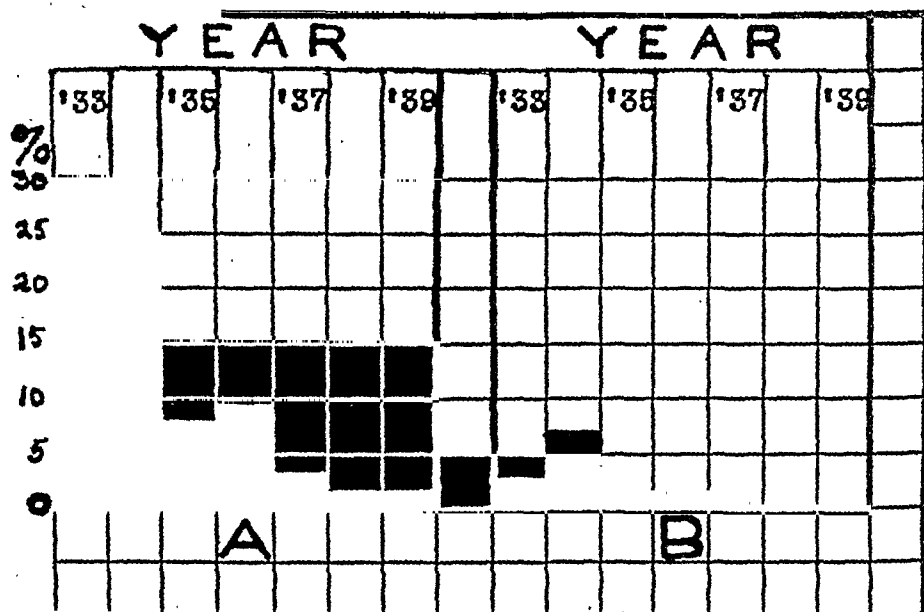
The other opportunity for observing the effect of authorized *H. pertussis* vaccine injections in young infants occurred in 1936 when Dr. Herman N. Bundesen, Chicago Commissioner of Health, requested the injection of alternate infants between the second and sixth months of life at the four welfare stations where the highest mortality from pertussis had occurred. During that summer 1,071 infants were injected. One year later, the parents of 758 were located; the remainder had moved without leaving an address. Twelve of these infants had developed whooping cough and 8 had been exposed and escaped. Of the 998 unvaccinated control infants in the same welfare stations, the parents of 757 were located. Eleven of these infants had developed whooping cough and 2 had been exposed without developing the disease.

From Table II it will be seen that the attack rate was not reduced by the injection of vaccine before the sixth month of

TABLE II
Data from Four Chicago Health Department Welfare Stations

Group	Number of infants	Number located 1 year later	Had whooping cough within the year	Attack rate (per cent)
Controls (no vaccine) Total dosage 8 cc. authorized	998	757	11	1.45
<i>H. Pertussis</i> vaccine	1071	758	12	1.58

of the vaccine made in that year, for not until 1935 was a gross error in vaccine production discovered and rectified. Text-Figure 1 shows the attack rate arranged according to the years in which the "Cradle" infants (A) and the older infants (B) were injected. Although the attack rate of each group decreased after 1935, that of the "Cradle" group remained the higher of the two. Of the 91 "Cradle" infants who later developed pertussis, 34 had



TEXT-FIGURE 1. Percentage of pertussis (1933 to January 1, 1940). A, injected before 3 months; B, injected after 7 months.

had 1934 vaccine; of the 3 privately injected Geneva infants who contracted the disease, 2 had had 1934 vaccine; of the 20 Evanston clinic infants, 17 had had 1934 vaccine; and of the 30 privately injected Evanston infants, 22 had had 1934 vaccine. Of the children injected after the seventh month who later developed pertussis, over 85 per cent were injected with 1934 vaccine. No serious complication and no death from whooping cough has been reported in any injected child who subsequently developed the disease.

On 89 "Cradle" infants 123 complement-fixation tests were performed* from 1 to 12 weeks after the final injection of vac-

* At first by Mr. F. G. Jones of Eli Lilly Laboratory; more recently by Mrs. Eva Markley of the Evanston Hospital Whooping Cough Research Laboratory.

life. The attack rate of 1.58 per cent at the end of 1 year was as high as that at the end of 7 years for all groups injected after the seventh month of life. Furthermore, the attack rate in the welfare clinic group, injected between the second and sixth months, was as high as that of the "Cradle" group, injected between the second and third months.

DISCUSSION

Although some infants at the age of 3 months possess the power to elaborate the specific antibody from hypodermically injected authorized *H. pertussis* vaccine, others seem to lack this power. Most of the "Cradle" infants injected after 1934, who developed pertussis later, had it in a mild form. No death from whooping cough has been recorded to date in any child who developed the disease after injection with authorized *H. pertussis* vaccine.

SUMMARY

Pertussis developed in 14.84 per cent of the Geneva control children who had had no vaccine; in 8.19 per cent of the "Cradle" children injected before the third month of life; in 2.48 per cent of the Evanston private patients injected after the sixth month of life; and in 1.44 per cent of the Geneva children injected after the seventh month of life.

CONCLUSIONS

1. Pertussis occurred seven times more frequently in children injected before the third month of life than in children injected after the seventh month.
2. For active immunization authorized *H. pertussis* vaccine should be injected after the seventh month of life.

more bright yellow corpora lutea, ranging in size from 1 or 2 mm. to about 1.5 or 2.0 cm. in diameter. These could be shelled out easily, the operation effecting a fairly sharp separation. The greatest difficulty was experienced with the small corpora lutea in that they were not easy to find or to separate cleanly. Many of these were revealed and made accessible for removal by cutting the ovary into thin slices.

The corpora lutea and the remaining ovarian tissue were separately ground in a meat grinder, placed in bottles containing about ten volumes of 95 per cent alcohol, and kept in the incubator 10 to 14 days. After this period the alcoholic solutions were filtered off while warm. On cooling to room temperature a white precipitate formed, most of which could be redissolved by adding alcohol. The extract of the corpora lutea was clear, deep yellow and contained 0.925 per cent solids. That of the ovary was a clear, straw-colored solution containing 0.714 per cent solids. Alcoholic extracts of other organs used in these experiments were prepared in a similar manner.

For the preparation of antigens for complement-fixation tests, measured quantities of the extracts containing known amounts of solids were evaporated to dryness. The residues were triturated in saline and made up to such a volume that 0.5 cc. contained 1 mg. of lipid. From this stock emulsion, dilutions were made. It has been found that equally active antigens can be prepared by adding measured quantities of the alcoholic extracts directly to saline. Such preparations should be boiled to remove most of the alcohol, the volume lost by evaporation being replaced with distilled water. The relative volumes of extract and saline used are chosen to give the proper concentration of lipoids.

The antisera of brain and testis used in these experiments were obtained from rabbits given repeated intravenous injections of suspensions in saline of the finely ground fresh organs. Anti-corpus luteum sera were from rabbits injected repeatedly and intravenously with an emulsion of the lipid from beef corpora lutea mixed with horse serum.

The results of complement-fixation reactions of antisera of beef brain, testis and corpus luteum with alcoholic extracts of various beef organs indicate the antigenic similarities and differences among the lipoids of the organs (Table I). No reactions

THE ANTIGENIC RELATIONSHIP OF ALCOHOL-SOLUBLE SUBSTANCES OF CORPUS LUTEUM TO THOSE OF TESTIS AND BRAIN*

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In a previous communication¹ it was shown that alcohol-soluble substances from the testis have antigenic properties very closely related to those from brain. An antiserum for lipoids of beef brain will give complement-fixation reactions with lipoids of beef testis and an antiserum for beef testis will react with lipoids of beef brain. Carefully conducted quantitative tests show that, with complement-fixation reactions at least, the alcohol-soluble antigens of these two-organs are indistinguishable.

Not only have beef brain and testis an antigenic reactivity in common with each other but also in common with brain and testis of all other species tested, including the rabbit which was the source of the antisera used in the experiments. These organs, therefore, lack species specificity but have a specificity that is presumably based on some common constituent present in brain and testis of various species. These facts fulfilled the conditions for iso-antigenicity, a property that has been demonstrated for brain² but not as yet for the testis, although there is no reason to believe that the experiments demonstrating iso-antigenicity for brain will give different results when performed with the antigenically related testis.

Since the ovary is the female homologue of the testis, the question arises as to whether the alcohol-soluble substances of the female organ share the antigenic specificity of the male organ. The present report concerns this question and deals with complement-fixation reactions between anti-brain, anti-testis and anti-corpus luteum sera and homologous and heterologous organ extracts.

The material used to prepare ovarian alcoholic extracts was a large number of fresh glands obtained from slaughtered beef. The ovaries were washed free of blood and the small amount of adventitial tissue trimmed off. Each gland contained one or

* Received for publication May 3, 1941.

with any of the antiserums were obtained with extracts of liver, kidney, heart, lung or spleen. The extract of ovary from which corpus luteum tissue was removed gave either no reaction or weak reactions. In contrast, the extracts of brain, testis and corpus luteum gave strong reactions with the homologous as well as with heterologous antiserums. It is possible that the weak reactions of the ovarian extract were due to an incomplete removal of corpora lutea from the ovaries in the preparation of the extract.

It is thus seen that brain lipoids, believed at first to be markedly organ-specific,³ have a common reactivity with those of at least two other organs. The possibility also exists that comparisons with the lipoids of other organs not included in these experiments, such as the adrenal, pituitary and thyroid, will disclose still other antigens that have antigenic properties in common with brain lipoids.

Another similarity of corpus luteum lipid to lipid of brain and testis is its lack of species specificity as indicated by the positive reactions obtained between an anti-human brain serum and beef corpus luteum lipid, and between anti-beef corpus luteum serum and sheep brain lipid (Table II).

TABLE II

Reaction of Beef Corpus Luteum Extract with a Heterologous Brain Antiserum and of Beef Corpus Luteum Antiserum with a Heterologous Brain Extract

Extract of	Antiserum for	Milligrams of alcoholic extract residue									
		1.0	0.6	0.4	0.2	0.1	0.06	0.04	0.02	0.01	0.006
Beef corpus luteum	Human brain	o	o	o	o	o	o	+	++	+++	++++
Sheep brain	Beef corpus luteum	o	o	o	o	o	o	o	++	+++	++++

o = absence of hemolysis and complete fixation of complement; ++++ = complete hemolysis and no fixation of complement; +, ++, +++ = various degrees of hemolysis and fixation of complement between o and ++++.

It is believed that the cross reactions between brain, testis and corpus luteum are due to the presence in their alcoholic extracts of a common antigen whose chemical nature is as yet unknown. Cholesterol, a lipid found in most tissues, does not seem to be the active element since its concentration in the alcoholic extracts of organs used in these experiments is not consistent with their

TABLE I
Reactions of Alcoholic Extracts of Beef Organs with Antiserums for Beef Brain,
Testis and Corpus Luteum

Beef organ extracts	Beef organ antisera	Milligrams of alcoholic extract residue									
		1.0	0.6	0.4	0.2	0.1	0.06	0.04	0.02	0.01	0.006
Corpus luteum	Corpus luteum	o	o	o	o	o	o	o	+	+	+
	Testis	o	o	o	o	o	o	o	+	+	+
Testis	Brain	o	o	o	o	o	o	o	+	+	+
	Corpus luteum	o	o	o	o	o	o	o	+	+	+
Brain	Testis	o	o	o	o	o	o	o	+	+	+
	Brain	o	o	o	o	o	o	o	+	+	+
Ovary	Corpus luteum	+	+	+	+	+	+	+	+	+	+
	Testis	+	+	+	+	+	+	+	+	+	+
Liver	Brain	+	+	+	+	+	+	+	+	+	+
	Corpus luteum	+	+	+	+	+	+	+	+	+	+
Kidney	Testis	+	+	+	+	+	+	+	+	+	+
	Brain	+	+	+	+	+	+	+	+	+	+
Heart	Corpus luteum	+	+	+	+	+	+	+	+	+	+
	Testis	+	+	+	+	+	+	+	+	+	+
Lung	Brain	+	+	+	+	+	+	+	+	+	+
	Corpus luteum	+	+	+	+	+	+	+	+	+	+
Spleen	Testis	+	+	+	+	+	+	+	+	+	+
	Brain	+	+	+	+	+	+	+	+	+	+

The details of the technic used in performing the complement-fixation tests are described in a former article.⁴
o = absence of hemolysis and complete fixation of complement; +, +, + = complete hemolysis and no fixation
of complement; +, +, + = various degrees of hemolysis and fixation of complement between o and +, +, +.

The antiserum for beef corpus luteum, in addition to reacting with alcoholic extracts of beef corpus luteum, will also react with extracts of beef brain and beef testis but not with those of liver, kidney, heart, lung or spleen.

An anti-human brain serum will react with an alcoholic extract of corpus luteum and an anti-beef corpus luteum serum will react with an alcoholic extract of sheep brain.

The cross reactions between the alcoholic extracts of brain, testis and corpus luteum are apparently not accounted for by the cholesterol present in the extracts.

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antigenic relationships. Cholesterol in each organ extract was determined colorimetrically by the Liebermann-Burchard reaction and expressed in percentages of the solid matter in the extracts. The figures, given in Table III, show that the brain extract contains approximately five times as much cholesterol as do the extracts of testis and corpus luteum, yet brain, testis and corpus luteum are antigenically equivalent as far as the reactions of their lipoids with antiserums for brain, testis and corpus luteum are concerned. The extract of ovary, which gave a feeble reaction at best with these antiserums, contains more cholesterol than the corpus luteum extract. The kidney extract contains more cholesterol than either testis or corpus luteum extract but gives entirely negative reactions with the antiserums tested. Moreover, the extracts of brain, testis and corpus luteum from which cholesterol is removed by precipitation with digitonin retain unaltered their antigenic relationship.

The fact that corpus luteum and testis are sex glands might indicate that sex hormones or their derivatives are concerned with their own antigenic relationship, but would not explain their relationship to brain unless this organ could also be shown to contain sex hormones or chemically similar substances.

TABLE III
Cholesterol Contained in Alcoholic Extracts of Beef Organs

Alcoholic extract of	Amount of solids per 100 cc.	Percentage of cholesterol in the solid fraction
Brain	1.211	25.19
Corpus luteum	0.926	5.22
Testis	0.543	4.35
Ovary	0.714	8.98
Liver	1.557	3.31
Kidney	1.082	7.21
Heart	0.746	3.12
Lungs	0.224	2.89
Spleen	1.444	5.68

CONCLUSIONS

The alcoholic extract of beef corpus luteum gives complement-fixation reactions with antiserums for beef corpus luteum, beef testis and beef brain.

The alcoholic extract of beef ovaries from which corpora lutea have been removed as completely as possible gives negligible reactions with the above antiserums.

composed of many fine nerve fibrils and nerve endings in the epithelium. He noted multipolar cells similar to those described by Köstlin, but did not regard them as ganglion cells because they lacked connection with nerve fibers. v. Gawronsky¹² (1894), in Golgi preparations, interpreted cells in the uterine wall as nerve structures. He also described bundles of nerves in the myometrium, which divided into fascicles under the endometrium and passed parallel to its surface. Fine branches from these fascicles ended in nodes in the endometrium. He stressed the presence of a rich nerve supply of the blood vessels.

Herlitzka¹³ in 1897 described three varieties of nerves in the uterine wall: (1) unmyelinated fibers to the blood vessels, (2) cerebrospinal nerves with Ranvier's nodes ending intracellularly and (3) myelinated fibers extending to the smooth muscle. Each system seemed independent. He described multipolar cells resembling ganglion cells in the uterus, entirely unconnected with the uterine nerves. Labhardt¹⁴ (1906) found no nerve structures in the endometrium, but noted nerve fibrils ending in muscle sheaths, insufficient to supply each muscle cell. La Torre,¹⁵ using the Cajal method, concluded that the intra-uterine nerves were unmyelinated. Hoogkamer¹⁶ in 1913 described nerve endings in the endometrium and fine fibrils extending between the epithelial cells. Though unable to demonstrate intracellular nerve endings, he found a variety of nerve cells in the uterus which suggested the presence of an extensive ganglion cell apparatus in the myometrium and mucosa. Dahl¹⁷ in 1916 demonstrated myelinated and unmyelinated nerves among the muscle fibers and about the blood vessels. The external os, according to his statements, receives only fine unmyelinated fibers, and the cervical muscle has a rich supply of both varieties of nerves. Dahl described cone-shaped enlargements in the termination of the nerves at the muscle fibers. He observed nerve bundles in the mucosa but no connections with the epithelium, nor could he demonstrate nerve cells in the uterine tissues. Oudendal¹⁸ in 1922 described small ganglion cells in the uterine wall. Mabuchi¹⁹ (1924) observed small spindle-shaped nerve endings in the myometrium, but no nerve elements in the endometrium. He concluded that stimulation passes directly from one muscle cell to another. Gemmell,²⁰ Fleming^{21,22} and Naiditsch²³ did not find

THE INTRINSIC NERVES OF THE IMMATURE HUMAN UTERUS*

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Certain phases of the intrinsic nervous tissue structures of the uterus have been investigated, but many of the finer details have not been explored or have been described without adequate confirmation.

LITERATURE

Remak¹ in 1840, according to Davis,² was the first to demonstrate histologically the presence of nerve fibers in the uterine tissues. He described myelinated nerves in the uterus of pregnant animals and unmyelinated nerves in nonpregnant animals. He did not observe nerve cells in the uterus. Many reports cite Lee³ (1841) as the first to demonstrate nerves in the human uterus. For several years there was an acrimonious correspondence between Lee⁴ and Beck⁵ over the accuracy of the former's observation.

Kilian^{6,7} in 1851 described myelinated nerve fibers in the uterus. Their distribution was uniform except in the cervix, where the supply was more abundant. He traced the nerves to the mucosal lining. Frankenhäuser⁸ in 1867 reported myelinated and unmyelinated nerve fibers in the uterus, the former supplying the blood vessels, the latter passing to the smooth muscle and sending small branches into the cells of the mucosal epithelial lining. Patenko⁹ in 1880 described a fine plexus of unmyelinated nerves in the submucosa from which fine fibrils extended to the epithelium. He also reported nerve cells along the nerve fibers in the muscularis. Köstlin¹⁰ (1894) observed multipolar cells in the uterus, similar to ganglion cells, but not connected with nerve trunks. He described a fine nerve plexus in the mucosa of the uterus with delicate filaments extending into the epithelium and ending in small nodes in the individual cells. Clivio¹¹ in 1894 reported intramuscular and submucous plexuses

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MATERIAL AND METHODS

The preceding review demonstrates the limited accurate information concerning the intrinsic nervous tissue structures of the uterus and the divergence of opinions concerning the anatomic details. Apparently one of the main obstacles has been the inadequacy of the staining methods used, none being sufficiently differential for the nerve elements.

A study of the nervous tissues of the uterus was initiated by applying to immature uteri the methods used in other studies of the peripheral nervous system by Masson²⁷ and Popoff.²⁸ Uteri from four infants constituted the material: one was from a fetus about 3 months premature; the second from an infant 4 months of age; the third from a child 2 years of age; the fourth from a child of 9 years. They were removed with contiguous structures such as broad ligaments, fallopian tubes, vagina and regional connective tissues, and fixed in Bouin's solution. Each uterus was embedded in paraffin and sectioned serially at 6 to 10 μ . Four to eight sections were mounted on each slide. As every second or third slide was stained, depending upon the number of sections on a slide, the gaps of unstained sections did not exceed 80 μ . All sections of the uterus from the child 9 years of age were stained. Goldner's²⁹ modification of Masson's trichrome stain was used. Sections of a gasserian ganglion of an adult were used as controls in the staining procedures. The axis cylinders of the nerve fibers stained red. Myelin, if present, was dissolved to leave a clear space about the axis cylinder. The supporting protoplasmic network in this space stained a faint purple-red. All collagenous fibers stained light green. The fibrous connective tissue nuclei stained dark blue, the red blood cells orange-red and the smooth muscle fibers of the uterus brick-red.

OBSERVATIONS

The general distribution of the nerves was similar in all uteri examined. The large nerves approached the uterus on both sides lateral to the cervix at and slightly above the level of the vaginal cul-de-sac. They were accompanied by many large blood vessels and were surrounded by loose fibrous connective tissue. At various levels corresponding to the upper part of the cervical canal and lateral to the internal os, these large nerve trunks had fusi-

nerve cells in the uterus. Fleming concluded that the control of uterine movements is maintained by the intramuscular neurons.

Davis² in 1933, using the intravital and supravital methylene blue method, or modifications of the methods of Cajal, Levaditi, Bielschowsky, and Prince, demonstrated myelinated and unmyelinated nerve fibers in the uterine tissues arising from nerve bundles of considerable size at the periphery. In the body of the uterus the nerve fibers were demonstrated among and parallel to the smooth muscle fibers and about the capillary blood vessels. Fine unmyelinated nerves were found in the cervix beneath the epithelium of the cervical canal and of the vaginal portion of the cervix. Davis was unable to find nerve fibers beneath the mucosa of the corpus uteri nor endings within the epithelium of any part of the organ. He emphasized the preponderance of nerves around the capillaries but failed to observe nerve endings in the vascular endothelium. He observed the subepithelial nerves below the vaginal portion of the cervix and considered the complicated plexus containing many spidery ganglion cells to be associated intimately with sensation and probably a part of the cerebrospinal system. Davis found no ganglion cells regularly in the substance of the uterus except in this portion of the cervix. He regarded the microganglia alongside the cervix as sufficient to perform the so-called automatic action in the separated uterus.

Keiffer²⁴⁻²⁶ in 1934-35, using the method of Bielschowsky, modified by Reumont and himself, described macroganglia and microganglia in the retrosphincteric connective tissue of the lower uterine segment. This included the sphincter with the mucosa of the internal orifice and all of the connective tissue surrounding this sphincter for a height of about 5 mm. and generally extending to the vaginal cul-de-sac. The ganglion cells apparently correspond to those described by others in the so-called cervical ganglion. Isolated nerve cells within the uterine wall appeared to be unipolar. Keiffer described several structures in the retrosphincteric connective tissue and within the uterine wall which he considered to be sensory corpuscles or nerve receptors. They appeared to be specially adapted to the physiology of the muscles because of their location between interwoven muscle bands. Others, associated with blood vessels, were regarded as chemical receptors.

none was within the uterine wall. A few cells in the course of some of the smaller nerve trunks in the body portion of the uterus resembled in structure and staining qualities those in the cervical ganglia. Other similar cells, perhaps ganglion cells, were at the bifurcation of small nerve trunks in the body portion of the uterus. These nerve trunks at the levels of bifurcation became more cellular. In the open part of the Y-shaped structure formed, there were several oval, red-purple nuclei with considerable chromatin and a small nucleolus surrounded by an irregular homogeneous purple cytoplasm. The appearance and staining qualities of these cells resembled nerve cells in the cervical ganglion but, not being found regularly, their interpretation as ganglion cells is doubtful.

COMMENT

Some problems confronting an investigation of the intrinsic nerves of the human uterus should be mentioned briefly. The first is of a technical nature and concerns tissue stains and the material used. Many of the staining methods, such as the silver and methylene blue methods, although excellent for demonstrating cellular detail, do not differentiate the various tissues, nor are they conveniently adaptable to serial sections. Goldner's modification of Masson's trichrome stain may be used with serial sections and gives excellent differentiation of the tissues, despite some loss of the finer cellular details. In the uterine tissues examined, the brick-red color of the smooth muscle fibers contrasted with the light green of the fibrous tissue. The smooth muscle cells in the walls of the blood vessels were recognized easily even in tangential sections. They were not confused with nerves longitudinally cut which have wavy green lines and slender purple sheath cells. Difficulties in distinguishing nerves from blood vessels occur with the smallest capillaries and single nerve fibrils. A single red blood cell within the lumen of such a capillary occasionally may be orange-red as is a single axis cylinder. Other difficulties in the interpretation of these structures usually can be avoided by tracing them in serial sections. The infantile uterus is suitable especially for serial sections. The principal disadvantages in its use are the immature development, or the slight alteration of staining qualities of the nervous tissue and

form dilatations with many small and medium-sized ganglion cells. Most of the nuclei were ovoid or slightly irregular, without a clearly demonstrated nucleolus. Many of the large nerve trunks entered the wall of the cervix with large blood vessels. The larger nerve trunks seemed to parallel the cavum of the uterus. Smaller branches from these trunks extended at angles toward the cavum of the cervix. In the cellular submucosa supporting the epithelial lining of the cervix these nerves branched into numerous small interlacing twigs usually composed of only one axis cylinder just beneath the columnar lining. Fibers did not extend into the epithelial cells. This fine nerve network apparently corresponds to the subepithelial plexus of the cervix described by Davis.²

Most of the large nerve trunks, deep within the wall of the cervix, extended along the long axis of the uterus into the body portion. Others, accompanied by large blood vessels, passed along the lateral side of the uterus in the tissues corresponding to the attachment of the broad ligament to higher levels of the corpus of the uterus. Some of these gradually passed through the outer layers of the myometrium to the anterior or posterior surface of the body of the uterus, then into the deeper portions of the myometrium. The number of large nerve trunks decreased progressively toward the fundus. The lamina propria or submucosa of the body of the uterus in the material studied did not have the extensively branching network of fine nerve fibers present in the submucosa of the cervix. Many small nerve fibers could be traced into the submucosa but not into the epithelial lining cells.

In all uteri examined, close association of the nerves and blood vessels existed. This was indicated by the proximity of the large nerves and blood vessels as they entered the uterine wall, often in the same connective tissue bundle. The relationship was maintained occasionally even in the smaller capillaries and nerve fibrils. In these small uteri the endings of axis cylinders in the walls of the blood vessels could not be demonstrated. In some places fibrils seemed to branch from a nearby nerve and to encircle small blood vessels.

Although numerous ganglion cells were present in the region of the so-called cervical ganglia on the lateral sides of the cervix,

were found which could be interpreted as sensory corpuscles within the uterine wall or nearby connective tissues.

NOTE: Since the completion of this report a study of nerves in the adult human endometrium has been accepted by the *Archives of Pathology* for publication. The nerve trunks in the myometrium beneath the endometrium contained mainly unmyelinated fibers and also one or several myelinated fibers.

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the failure to demonstrate the axis cylinders with the same precision as in the gasserian ganglion of an adult.

The presence of nerve cells or other structures which may initiate or maintain the so-called automatic action and tone of the uterine musculature is not established. Davis² believed that sufficient microganglia are distributed along the cervix to provide the mechanism necessary for the so-called automatic action in the isolated uterus. Keiffer²⁴⁻²⁶ considered the nerve cells in the retrosphincteric connective tissue in the lower segment, apparently the cervical ganglia, the reflex center for the uterus. The experiments of Fleming^{21, 22} and Kaminester and Reynolds,³⁰ demonstrating that the tone and movements of the uterus are independent of the cervix and cervical ganglia, provide evidence that the origin of the controlling nervous impulses is above the level of the cervix and cervical ganglion. Some authors have described nerve cells within the wall of the uterus from which such impulses could arise. Others have failed to corroborate this observation.

SUMMARY

The distribution of the nerves in the infantile uteri examined resembles that described by other investigators. Nerves pass from the cervical ganglia on the lateral sides of the cervix into the wall of the uterus accompanied by large blood vessels. The larger nerve trunks extend parallel to the long axis of the uterus and lie deep within the myometrium. Small branches extend from these trunks to the endometrium, without entering the epithelial lining cells. They form an intricate network or plexus in the lamina propria of the cervical canal. The lamina propria of the body of the uterus has fewer nerves than the cervix. The nerve trunks to the upper portion of the body of the uterus ascend in the connective tissue of the broad ligaments or in the superficial layers of the myometrium at the periphery of the uterine wall. The number of nerve trunks diminishes progressively toward the fundus of the uterus. The significance of the close anatomic association of the nerves and blood vessels within the uterine wall is not clear. A similar relationship exists between the nerves and blood vessels in other tissues of the body. Nerve endings in the vascular walls were not demonstrated. No tissues

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RESULTS

Amyloid was found in the kidneys, liver or spleen (or in several of these organs) in 104 of the 181 rabbits injected with bacteria, or 57 per cent. In general, rabbits injected with strains of virulent hemolytic streptococci, Friedländer's bacilli or pneumococci developed amyloid sooner and more extensively than animals given the relatively avirulent green-producing streptococci or other organisms long after their isolation from human cases. In the case of staphylococci, septicopyemia killed the animals in too short a period to permit development of amyloidosis. The dosage used may have been too large, for other investigators¹⁻³ have produced amyloidosis in rabbits with this organism. No other organism employed failed to bring about the deposition of amyloid in some rabbits.

The histology of experimental amyloidosis has been repeatedly described and we have alluded to it in earlier preliminary reports.^{4,5} In a large majority of rabbits coming to autopsy before amyloidosis has had time to develop, marked lymphoid and reticular cell hyperplasia of the splenic follicles has been observed, often with numerous mitotic figures. The amyloid was always extracellular.

Factors in the Distribution of Amyloid

The localization of amyloid was characteristic. In the kidney, amyloid was found only in the glomerular tuft and along the medullary capillaries. In the spleen, amyloid was usually deposited in the periphery of the follicle, extending inward and outward as it increased. The follicular arterioles were only slightly involved. In the liver, amyloid was observed about the peripheral sinusoids, extending centrally and producing the typical atrophy. The adrenal gland exhibited amyloid around capillaries in the zona reticularis near the medulla, spreading later toward the zona fasciculata. In no case was the entire adrenal cortex infiltrated nor extensive atrophy of tissue produced. Amyloid was never found in the myocardium, lungs, thymus or aorta. It was sometimes seen in the tail of the pancreas, involving the capillaries of both the ordinary and islet parenchyma. A few observations of the stomach and intestines showed amyloid about the capillaries of the mucosa.

SOME FACTORS IN THE DEVELOPMENT, LOCALIZATION AND REABSORPTION OF EXPERIMENTAL AMYLOIDOSIS IN THE RABBIT*

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Certain aspects of the problem of amyloidosis have engaged our attention for a number of years. This paper is a summary of our findings, and is dedicated to a great teacher and investigator of pathology whose manifold interests also include experimental amyloidosis.

METHODS

Healthy rabbits, weighing between 1500 and 2000 gm. and kept on an adequate mixed diet, were injected intravenously with strains of hemolytic streptococci freshly isolated from the upper respiratory passages of patients with acute glomerulonephritis. Scarlet fever streptococci, green-producing streptococci from the respiratory passages or urines of patients with chronic nephritis and systemic lupus erythematosus, pneumococci of types I and III, and a Friedländer bacillus were also used, along with some other strains, including a hemolytic streptococcus of canine tonsillar and endocardial origin. All organisms were grown in blood broth.

A series of rabbits was also injected with scarlet fever anti-toxin, horse serum, rabbit serum, rabbit plasma albumin and rabbit plasma globulin.

Material for histologic study was fixed in a 10 per cent aqueous solution of formaldehyde and in Zenker's solution and stained with Congo red for amyloid and with hematoxylin and eosin for general purposes. Ordinarily, kidney, liver, spleen, adrenal and myocardium were sectioned; occasionally, other organs.

Determinations of plasma proteins, blood urea and nonprotein nitrogen, and plasma cholesterol were made by the usual clinical microchemical methods. Albuminuria was estimated with the heat and acetic acid test and at times by quantitative chemical analysis.

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showing predominance of splenic amyloid in spite of a total experimental period of 12 to 14 months, the time elapsed from the beginning of the second injection course to death was $4\frac{3}{4}$ and 2 months, respectively, or long enough to permit the deposition of amyloid. In the 4 rabbits in group (c), 3 had total experimental periods of 10 to 15 months, with the second experimental periods of 1, $4\frac{1}{2}$ and 5 months, respectively. In this group either the first or the second injection courses could have produced the amyloid in 2 of the 3 rabbits. On the other hand, 3 rabbits in group (d) had total experimental periods of 5, 17 and 28 months, respectively. In the latter 2 rabbits there was no amyloid in the spleen, but the second experimental periods lasted 10 and 16 months, respectively, or long enough to explain the predominantly renal amyloid.

Factors in the Development of Amyloidosis

The duration of bacterial injections seems to have little relation to the development of amyloidosis except for the longer period required in the case of avirulent strains. In our earlier experiments,⁴ it was found that freshly isolated and virulent strains of hemolytic streptococci from cases of acute nephritis produced albuminuria (renal amyloid) much sooner than did the less virulent or older strains. Without frequent biopsies on the spleen and in the absence of albuminuria it is impossible to determine the exact onset of amyloidosis. However, in 17 rabbits with well developed amyloidosis the entire injection period extended over 3 weeks or less, in 6 animals for only 3 to 4 days. Yet in this group the total experimental period was $1\frac{1}{2}$ to 3 months in 4 out of 5 rabbits injected with freshly isolated strains of "nephritic" hemolytic streptococci, and from 12 to 26 months in all 9 rabbits injected with freshly isolated strains of *Streptococcus viridans* or other avirulent organisms.

The rôle of chronic infection and suppuration was not clearly significant in our series of positive animals. Disregarding terminal infections in the lung, only a small percentage of our rabbits had vegetative endocarditis, suppurative arthritis, cholecystitis, pericholecystitis or hepatitis, or chronic pneumonitis. The rabbits without amyloid were infected to about the same extent as the positive animals. The etiologic significance of small infarcts in

The relative distribution of amyloid in the main sites of occurrence—kidney, spleen, liver and adrenal—was determined largely by the total length of the experimental period and the duration of survival after the injection period (Table I). Thus, grading the amount of amyloidosis on a basis of 4 plus as a maximum in each organ and classifying the rabbits into groups, the following distribution was obtained: (a) No amyloid in 80 rabbits living less than 7 weeks. There were a few animals without amyloid living longer periods, up to 38 months. (b) Moderate to considerable amyloid in the spleen (or liver) with little or none in the kidneys in 24 rabbits, of which 9 died in 4 to 8 weeks, 12 in 3 to 5 months, none in 6 to 11 months and 3 in 12 to 18 months. (c) Moderate to considerable amyloid in the spleen, kidneys and often the liver, less often in the adrenal, in 26 rabbits, of which none died prior to 8 weeks, 13 in 2½ to 6 months, 4 in 7 to 11 months and 9 in 12 to 24 months. (d) Moderate to considerable amyloid in the kidneys and often the adrenals, with little or none in the spleen or liver in 36 animals, of which 1 died in 2½ months, 1 in 5 months, 14 in 7 to 11 months and 20 in 12 to 30 months.

TABLE I

Distribution of Amyloid According to Duration of the Experimental Period

Period	Total No. of rabbits used	No. without amyloid	With generalized amyloidosis	With amyloid chiefly splenic, little or none in kidneys	With amyloid chiefly renal, little or none in spleen
1-2 mo.	77	68	0	9	0
3-6 mo.	31	4	13	12	2
7-11 mo.	20	2	4	0	14
12-30 mo.	35	3*	9†	3‡	20

Eighteen rabbits used in the experiment are not included in this table since, while they showed slight amounts of amyloid, there was too little to consider organ-distribution significant.

* 12-38 months.

† 12-24 months.

‡ 12-18 months.

|| 12-30 months.

Apparently, bacterial amyloidosis develops first in the spleen and liver and later in the kidneys with subsequent diminution or disappearance of splenic and hepatic amyloid but an increase in renal and, probably, in adrenal amyloid.

In 10 rabbits bacteria were injected in two separate courses, 8 to 10 months apart in 6 animals and from 1½ to 3½ months apart in 4 animals. In 2 out of the 3 rabbits in the group (b)

cent, 5 being over 2.93 gm. per cent. These globulin figures are slightly lower than those we⁶ reported on another series of 46 rabbits with an average control level of 1.86 ± 0.37 gm. per cent.

During the period of bacterial injection, if not less than 3 weeks, the plasma globulin rose to 3 gm. per cent or higher in 34 out of 40 rabbits studied. Elevated plasma globulin was maintained during the longer injection courses.

The plasma globulin values after the period of bacterial injection varied considerably in different rabbits and often in the same rabbit. In 15 animals, the plasma globulin exceeded 2.5 gm. per cent during 2 months or longer, often reaching levels of 3.5 to 4 gm. per cent. However, in 14 rabbits the plasma globulin remained for several months below 2.5 gm., often below 2.0 gm. per cent. No clear difference in the degree or distribution of amyloidosis was found in these two groups, but 5 out of 8 rabbits without amyloid had low plasma globulins. Finally, a number of animals showed irregular fluctuations over a period of months. In the rabbits receiving two courses of bacteria, the second injection period usually was followed by a sharper rise in plasma globulin than the first. Albuminuria, sufficient to lower the plasma albumin to 2.5 gm. per cent or less, was usually associated with an elevation of plasma globulin to 2.5 gm. per cent or more, apart from the possible influence of bacterial injections. The total plasma protein usually reached 7 to 8 gm. per cent when the globulin fraction increased, except in cases of low plasma albumin secondary to albuminuria, when the plasma proteins fell to 5 or 4 gm. per cent.

The plasma globulin was also elevated by the repeated intravenous injection of scarlet fever antitoxin (horse). Of 5 rabbits injected over a maximum period of $1\frac{1}{2}$ months, 3 died of pulmonary edema, 1 lived 10 months and 1 for 2 years (after a single injection). None showed amyloid but all had marked hyperplasia of the spleen. The plasma globulin values in the 4 rabbits studied reached 3.36 to 5.94 gm. per cent during the period of injection, the rise beginning within a week. In the 2 rabbits that survived the injection period, the plasma globulin figures remained within normal levels.

Direct elevation of the rabbit's plasma globulin was made by intravenous injection of rabbit whole serum and rabbit plasma

the kidney seemed dubious. Rabbits with suppuration due to staphylococci died too soon to develop amyloidosis.

Reabsorption of Amyloid

Apart from direct biopsy evidence available in a small series of rabbits,⁵ strong indirect support of the reabsorption theory is given by the data on the distribution of amyloid in the kidneys, spleen, liver and adrenals in relation to the total experimental period (Table I). In the spleen, the process of reabsorption seems to be largely the result of invasion of leukocytes, polyblasts and capillaries from the adjacent pulp. The amyloid loses its staining power, is broken up into fragments and gradually disappears. Participation of foreign body giant cells in this process^{2, 6-8} was limited to 3 animals although a few more showed giant cells, presumably megakaryocytes, unrelated to the amyloid masses. Following the reabsorption of splenic amyloid the follicles remained atrophic and at times definitely fibrotic. The liver showed little or no residual portal fibrosis. No clear instance of absorption of amyloid in the adrenal has been found in our series.

Evidence for reabsorption of renal amyloid in the rabbit has not appeared in our extensive study. Once the glomeruli and medullary capillaries have become moderately involved, tubular dilatation and degenerative changes set in with later atrophy and ultimate fibrosis. The kidneys usually remain large in spite of fibrosis because of the persistent amyloid, and weights two or three times the normal are a regular occurrence even in the late stages of renal disorganization. The rôle of tubular obstruction in parenchymal atrophy is important.

Experimental Hyperglobulinemia and Amyloidosis

This aspect of the problem has been studied in several ways. First of all, data have been obtained on the variations in the plasma proteins during and after the injection of bacteria in rabbits with or without amyloid. The average control plasma albumin in 73 rabbits was 4.28 ± 0.40 gm. per cent, with 11 values below 3.88 and 17 above 4.68 gm. per cent. The mean control plasma globulin in this series was 1.70 ± 0.41 gm. per cent, with 14 values below 1.29 gm. per cent and 11 values above 2.11 gm. per

Proteinuria

Albuminuria, if persistent, practically always indicated renal amyloidosis.^{4,5} In general, the degree and duration of proteinuria were proportional to the amount of glomerular amyloid. No albuminuria was found in rabbits with considerable amyloid in the spleen or liver but without renal amyloid. However, the absence of proteinuria did not exclude the presence of traces or small amounts of amyloid in the kidney, as was demonstrated in 7 rabbits. Following proteinuria, the plasma albumin fell to 2 gm. per cent or less, the plasma globulin often rose to 2.5 gm. per cent or more, the weight went down and the plasma cholesterol increased temporarily. In several animals the hypo-albuminemia caused transudation into the serous cavities.

COMMENT

Our experiments on some 200 rabbits have demonstrated the facility of production of amyloidosis in this animal by means of intravenous injection of bacteria. This is in agreement with the scattered results of previous investigators.^{1,2,10-12} The most rapid and most extensive amyloidosis has followed the use of hemolytic streptococci freshly isolated from inflamed upper respiratory passages and tonsils of individuals with acute, recurrent subacute, or active chronic glomerulonephritis. Other organisms have also proved effective in initiating amyloidosis when freshly isolated. After ageing on laboratory media, larger doses and more prolonged injections are necessary. In a great majority of our positive experiments, no focus of chronic suppuration or inflammation was found to account for the apparently progressive evolution of amyloidosis long after injections of bacteria had ceased and long after they must have disappeared from the animal body. In several rabbits, only three or four daily injections of bacteria sufficed to produce amyloidosis.

A constant and striking feature in this study was the change in distribution of amyloid with the lapse of experimental time. Splenic amyloid predominated markedly over renal amyloid in practically all of the rabbits coming to autopsy within 2 months of the onset of the experiments. Animals with periods of 2 to 6 months were very likely to show more or less uniform amyloidosis in the spleen, kidneys and liver. After 7 to 11 months the

globulin prepared in various ways. Five rabbits were given serum for periods up to 7 months. Three animals with a total of 1160 to 3000 cc. of serum during 5 to 7 months, with total experimental periods of 9, 16 and 19 months, showed slight to moderate amyloidosis in the kidneys at autopsy, although no amyloid was detected in either the kidney or portion of spleen removed at 5, 1½ and 5 months, respectively. Hyperplasia of the splenic follicles was found in the biopsy specimens. The plasma globulin in the 4 rabbits studied was maintained at 3 to 4 gm. per cent during the period of injection except when the amount of serum was reduced. The plasma albumin also rose so that the total protein reached 8 to 9 gm. per cent. When the serum was discontinued, the plasma globulin promptly fell to normal.

Rabbit plasma globulin was injected intravenously for periods of 1½ to 4 months in 6 rabbits. Considerable splenic or renal amyloid developed in 4 animals, a trace in 1 and none in 1. The definitely positive results were obtained with the more denatured globulin preparations. The plasma globulin rose during the injection period to 3 to 4 gm. per cent in all the rabbits, and persisted at a high level as long as globulin was given in adequate amounts. The highest plasma globulins, 4.6 to 5.9 gm. per cent, for periods of 1½ and 2½ months, were observed in the 2 animals with no amyloid. The plasma globulin fell to normal within a few weeks after the injection period except in the rabbits with persistent albuminuria due to renal amyloidosis. In 2 rabbits given the heat-denatured globulin many giant cells containing amyloid were seen in the spleen.

Dietary Experiments

A few experiments involving the injection of bacteria into rabbits kept on diets free from ascorbic acid or consisting of hay alone revealed no definite influence upon the production or course of amyloidosis. However, in 1 rabbit kept as a control on a hay diet for 10 months and surviving another 7 months on the regular diet, considerable amyloid was present in the kidneys without an obvious focus of inflammation. No amyloid has been observed in any control rabbit on the regular diet up to 3 years nor in rabbits used as blood donors.

results difficult to interpret, unless we assumed that the method of preparation of the serum proteins was such as to denature them for the rabbit. In that event the absence of amyloid in the 2 animals given the best globulin preparation is strong evidence against the simple assumption of hyperglobulinemia as a cause of amyloidosis. It has been reported,¹⁸ without details, that electro-ultrafiltered rabbit globulin produced neither amyloid nor precipitins in rabbits. More experiments are necessary, including identification of the plasma globulin during the formation of amyloid.

The more indirect attempts to correlate hyperglobulinemia and amyloidosis^{5,17,21} cannot, in our experience, lead to a conclusive decision. Whether amyloid is formed or not, the parenteral injection of antigen stimulates a rise in plasma globulin, relative or absolute. The plasma globulin level may be markedly increased or normal for months after the injection period in rabbits with or without amyloidosis. The low figures cannot be explained, as has been suggested,²¹ on the basis of proteinuria or hepatic amyloidosis. A second course of injections of bacteria or other antigen usually leads to a rapid rise in the plasma globulin level even in rabbits which failed to respond to the first series.

Some miscellaneous observations are of interest. The incidence of gross aortic medial necrosis, calcification and atheromatosis⁹ in the amyloid rabbits was 12 per cent, six times as high as in the nonamyloid and control groups. Anemia and loss of weight were frequent concomitants of the process of amyloidosis and were intensified during periods of considerable proteinuria. Anemia itself did not cause amyloidosis in a series of blood-donor rabbits. The spleen often contained many erythrocyte-laden and blood-pigment-laden macrophages. In rabbits with experimental periods of a year or longer, renal amyloidosis was usually associated with marked tubular atrophy and obstruction, fibrosis of the parenchyma and hyalinization of the glomeruli containing amyloid. In spite of this "contraction" of the kidneys, the weight still exceeded the normal because of the amyloid content. Amyloid was practically never found in any renal vessels other than the intraglomerular arterioles.

tendency increased for marked renal and adrenal amyloid with little or none in the spleen and liver. The apparent exceptions of behavior could be reasonably explained as the deposition of amyloid in response to a second, or more recent, course of injection of bacteria. In several instances biopsies of the spleen and kidney gave direct confirmation of the later change from predominantly splenic to predominantly renal amyloid.

These observations on the reabsorption of amyloid in the spleen and liver confirmed previous reports on reversibility of amyloidosis in these organs in the mouse,⁸ rabbit,^{2,3} horse⁷ and man.^{2,6,13,14} However, in spite of discontinuance of bacterial injections and the disappearance of splenic amyloid, renal amyloid not only remained but actually increased, ultimately causing disorganization of parenchyma and varying degrees of fibrosis and functional impairment. While the situation may be different in human renal amyloidosis, the literature is not convincing.^{6,13,14} Physiologically, conditions are more favorable for the reabsorption of amyloid in the spleen and liver than in the kidney.

There has been much speculation concerning the chemical nature of amyloid, the mechanism of its precipitation in the walls of arterioles and capillaries, and the relation of the processes of immunity, including hyperglobulinemia, to the pathogenesis of amyloidosis. A recent excellent study of the physical chemistry of human amyloid¹⁵ illustrates the difficulties involved. The rôle of chondroitin-sulfuric acid is still an intriguing problem.¹⁶ Such factors as hyperglobulinemia, the circulating or local precursors of amyloid, antigen-antibody precipitation and others have been discussed at some length by previous investigators.¹⁷⁻²¹ It is generally agreed that any type of prolonged cellular stimulation by foreign protein of external or internal origin leads to both hyperglobulinemia and amyloidosis in a highly susceptible animal, like the mouse or the rabbit, or the horse used for the production of immune sera.⁷ The apparent rarity of amyloidosis in individuals with kala-azar or lymphogranuloma inguinale, in which very high plasma globulin levels often occur, is difficult to understand.

Our direct attempt to test the theory of hyperglobulinemia as a cause of amyloidosis by injecting whole rabbit serum or concentrated rabbit plasma globulin in 10 rabbits yielded conflicting

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SUMMARY

1. Amyloidosis has been produced in a large series of rabbits by the injection of various bacteria from human sources.

2. Splenic and hepatic amyloid appear early, but can also disappear in time. Evidence of active reabsorption of amyloid is presented.

3. Renal amyloid develops later but tends to increase with time to the point of extreme disorganization and fibrosis of the parenchyma, and functional insufficiency. There is no evidence of absorption of renal amyloid in the rabbit.

4. Hyperglobulinemia, relative or absolute, is a constant finding during the longer periods of bacterial injection, but may or may not persist in the after period. Albuminuria may elevate the plasma globulin relatively.

5. Artificial hyperglobulinemia, the result of injections of rabbit serum or globulin, does not regularly produce amyloidosis. The positive results may be secondary to denaturation of the serum proteins. Presumably, other factors than hyperglobulinemia are necessary for the development of amyloidosis.

6. Amyloidosis may appear and progress in the absence of ordinary signs of inflammation or suppuration in the rabbit.

7. Gross aortic disease in the form of medial necrosis and calcification or atheroma is six times as prevalent in rabbits with amyloidosis as in the nonamyloid series. Atheroma is always associated with persistent hypercholesterolemia.

8. The pathogenesis of amyloidosis is not adequately explained by the prevailing theories and requires further investigation.

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